Feb 09, 2026

Materials and Methods for Cdk1 cooperates with EGFR–MAPK signalling to coordinate the final mitosis with neuronal fate specification in Drosophila

DOI
https://dx.doi.org/10.17504/protocols.io.5qpvo18d9g4o/v1

Fangxia Wang[1], Jin Man[1], Rusong Ding[1], Xue Xia[1], Hong Ran[1], Yaqi Sun[1], Qingyang Li[1], Kim Hou Chia[2], Daimo Li[1], Yusanjiang Abula[1], Hiroyuki Yamano[2], Yuu Kimata[1]

[1]ShanghaiTech University; [2]University College London Cancer Institute

Yuu Kimata: Corresponding author;

Cdk1 cooperates with E...

Yuu Kimata

OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.5qpvo18d9g4o/v1

Protocol Citation: Fangxia Wang, Jin Man, Rusong Ding, Xue Xia, Hong Ran, Yaqi Sun, Qingyang Li, Kim Hou Chia, Daimo Li, Yusanjiang Abula, Hiroyuki Yamano, Yuu Kimata 2026. Materials and Methods for Cdk1 cooperates with EGFR–MAPK signalling to coordinate the final mitosis with neuronal fate specification in Drosophila. **protocols.io**
https://dx.doi.org/10.17504/protocols.io.5qpvo18d9g4o/v1

Protocol status: Working
We use this protocol and it's working

Created: February 08, 2026

Last Modified: February 09, 2026

Protocol Integer ID: 242888

Keywords: drosophila precise coordination between cell cycle progression, universal mitotic kinase cdk1, drosophila precise coordination, targeting cell cycle regulator, drosophila eye imaginal, signalling pathway, cell cycle progression to neuronal specification, intrinsic cell cycle, cell cycle regulator, mitosis to prime mapk, ets family transcription factor pointed, function for cdk1, final mitosis with neuronal fate specification, cellular differentiation, coupling cell cycle progression, cell cycle progression, dependent transcription, loss of cdk1, transcriptional activity, methods for cdk1, high cdk1 activity, temporal integration of proliferation, prime mapk, essential for tissue development, mitosis, mapk, cdk1, final mitosis, transient repression of the proneural factor atonal, cooperation between core cell, neuronal fate specification

Funders Acknowledgements:
ShanghaiTech University start-up grant
Grant ID: 2018F0202-000-06
National Natural Science Foundation of China (NSFC) Mainshang Project
Grant ID: 32170746

Abstract

Precise coordination between cell cycle progression and cellular differentiation is essential for tissue development, yet the mechanisms that couple these processes remain poorly understood. Using the *Drosophila* eye imaginal disc as a model for spatial and temporal integration of proliferation and neuronal specification, we performed a systematic RNA interference and overexpression screen targeting cell cycle regulators. This screen identified the universal mitotic kinase Cdk1 as a key link between intrinsic cell cycle control and extrinsic EGFR–Ras–MAPK signalling. Loss of Cdk1 caused a selective block in EGFR-dependent R2/5 photoreceptor differentiation that could not be explained by G2 arrest or apoptosis, revealing a non-canonical, differentiation-specific function for Cdk1. Mechanistically, Cdk1 cooperates with MAPK signalling by converging on the ETS family transcription factor Pointed-P2 (PntP2), phosphorylating it at both shared and Cdk1-biased sites to regulate its transcriptional activity and nuclear localisation. PntP2 is expressed during the final mitosis preceding differentiation, coinciding with high Cdk1 activity and transient repression of the proneural factor Atonal, which promotes R8 fate. These findings support a model in which Cdk1 acts during the final mitosis to prime MAPK-dependent transcription and bias subsequent fate decisions, thereby coupling cell cycle progression to neuronal specification. Our study reveals a spatiotemporally orchestrated cooperation between core cell cycle machinery and developmental signalling pathways that synchronises proliferation with differentiation, a principle likely conserved across metazoan development.

Materials

Materials and Methods

Fly stocks and genetics

_Drosophila melanogaster stocks were maintained under standard conditions at 25 °C unless otherwise stated. RNA interference (RNAi) and transgene expression were driven using *ey-* or *Optix-Gal4* drivers to target retinal progenitors anterior to and within the morphogenetic furrow. For temporal control, Gal80ts was used where indicated. All experimental genotypes were generated by standard genetic crosses. For phenotypic analyses, sibling controls were analysed in parallel. A complete list of fly stocks and genotypes is provided in Supplementary Information.

Immunofluorescence and microscopy

Third instar larval eye imaginal discs were dissected in PBS, fixed in 4% paraformaldehyde, and processed for immunofluorescence using standard protocols. Primary antibodies included those recognising photoreceptor markers, phosphorylated Erk, and GFP. Alexa Fluor–conjugated secondary antibodies were used for detection. Nuclei were counterstained with DAPI. Images were acquired using a laser-scanning confocal microscope under identical acquisition settings for control and experimental samples. Unless otherwise indicated, single optical sections or defined z-projections were used consistently within each experiment.

Quantification and statistical analysis

Quantifications were performed on raw images using ImageJ. Regions of interest were defined based on anatomical landmarks such as the MF or photoreceptor clusters. For each experiment, multiple discs from independent animals were analysed. Statistical analyses were performed using GraphPad Prism. Data are presented as mean ± s.d. as indicated. Statistical significance was assessed using unpaired two-tailed t-tests or one-way ANOVA with appropriate post hoc tests. Exact sample sizes and statistical tests are specified in the figure legends.

RNA sequencing analysis of Drosophila eye-antennal discs and data availability

Eye imaginal discs were collected from control and experimental larvae, and total RNA was extracted using standard methods. Library preparation and sequencing were performed using Illumina platforms. Differentially expressed genes were identified using an adjusted false discovery rate (FDR) threshold of c 0.05. Bioinformatic analyses were performed using established pipelines as detailed in the Supplementary Information.

In vitro kinase assays

Recombinant PntP2 fragments were expressed and purified from *E. coli_. In vitro kinase assays were performed using active Cdk1–Cyclin complexes or activated Rolled kinase in the presence of ATP. Phosphorylation was assessed by immunoblotting using proline-directed phospho-specific antibodies or site-specific antibodies where*

indicated. For thiophosphorylation assays, ATPγS was used followed by alkylation and detection with an anti-thiophosphate ester antibody. These assays were used to assess kinase specificity and site selectivity. Detailed reaction conditions are provided in the Supplementary Information.

Resources and data availability

All *Drosophila* strains, antibodies, and other reagents used in this study, as well as additional data supporting the findings, are available from the corresponding author upon reasonable request. The RNA-seq data generated in this study have been deposited in the NCBI Gene Expression Omnibus (GEO) under accession number GSE312191. Raw FASTQ files and processed count matrices are publicly accessible.

Fly stocks and genetics

1 Maintain *Drosophila melanogaster* stocks under standard conditions at 25 °C unless otherwise stated. Use RNA interference (RNAi) and transgene expression driven by *ey-* or *Optix-Gal4* drivers to target retinal progenitors anterior to and within the morphogenetic furrow. For temporal control, use Gal80ts where indicated. Generate all experimental genotypes by standard genetic crosses. Analyze sibling controls in parallel for phenotypic analyses. Refer to Supplementary Information for a complete list of fly stocks and genotypes.

Immunofluorescence and microscopy

2 Dissect third instar larval eye imaginal discs in PBS, fix in 4% paraformaldehyde, and process for immunofluorescence using standard protocols. Use primary antibodies recognizing photoreceptor markers, phosphorylated Erk, and GFP. Detect with Alexa Fluor–conjugated secondary antibodies. Counterstain nuclei with DAPI. Acquire images using a laser-scanning confocal microscope under identical acquisition settings for control and experimental samples. Use single optical sections or defined z-projections consistently within each experiment unless otherwise indicated.

Quantification and statistical analysis

3 Perform quantifications on raw images using ImageJ. Define regions of interest based on anatomical landmarks such as the MF or photoreceptor clusters. Analyze multiple discs from independent animals for each experiment.
Perform statistical analyses using GraphPad Prism. Present data as mean ± s.d. Assess statistical significance using unpaired two-tailed t-tests or one-way ANOVA with appropriate post hoc tests. Specify exact sample sizes and statistical tests in the figure legends.

RNA sequencing analysis of Drosophila eye-antennal discs and data availability

4 Collect eye imaginal discs from control and experimental larvae, and extract total RNA using standard methods. Perform library preparation and sequencing using Illumina platforms. Identify differentially expressed genes using an adjusted false discovery rate (FDR) threshold of c 0.05. Conduct bioinformatic analyses using established pipelines as detailed in the Supplementary Information.

In vitro kinase assays

5 Express and purify recombinant PntP2 fragments from *E. coli_. Perform in vitro kinase assays using active Cdk1–Cyclin complexes or activated Rolled kinase in the presence of*

ATP. Assess phosphorylation by immunoblotting using proline-directed phospho-specific antibodies or site-specific antibodies where indicated. For thiophosphorylation assays, use ATPγS followed by alkylation and detection with an anti-thiophosphate ester antibody. Use these assays to assess kinase specificity and site selectivity. Detailed reaction conditions are provided in the Supplementary Information.

Resources and data availability

6 All *Drosophila* strains, antibodies, and other reagents used in this study, as well as additional data supporting the findings, are available from the corresponding author upon reasonable request. The RNA-seq data generated in this study have been deposited in the NCBI Gene Expression Omnibus (GEO) under accession number GSE312191. Raw FASTQ files and processed count matrices are publicly accessible.