

1. What mechanisms determine the number of cells that belong to stage 4?

The mechanisms that determine the number of cells in stage 4 are as follows:

- Differentiation into stage 4: This corresponds to the transcriptional activity of MyoD (at stage 3), which promotes myogenin expression in stage 4. The change in myogenin expression results from strain effects on signaling molecules (S) that cause MyoD to bind to the myogenin promoter, thereby increasing myogenin expression and transitioning cells into stage 4.
- Differentiation from stage 4 to stage 5: The combination of free myogenin in stage 4 and the action of strain-related signaling molecules causes myogenin to bind to the myosin heavy chain (MHC) promoter, increasing MHC gene expression and leading to the formation of more mature muscle cells.
- Cell death in stage 4: this mechanism removes cells from the stage 4 population.

2. What mechanisms determine the number of cells that belong to stage 3?

The mechanisms that determine the number of cells in stage 3 are as follows:

- Proliferation (self-renewal) in stage 3: a fraction of daughter cells remains in stage 3 after division (self-renewal)
- Differentiation into stage 3: stage 2 cells can divide, producing daughter cells that acquire enough MyoD to move into stage 3 (MyoD+).
- Differentiation into stage 4: stage 3 cells differentiate directly into stage 4 under the influence of MyoD. MyoD promotes myogenin expression (defining stage 4). When MyoD binds to the myogenin promoter, enhanced by the strain-generating signaling molecule S, it pushes these stage 3 cells into stage 4.
- Cell death in stage 3: some cells in stage 3 go into apoptosis, removing them from the stage 3 population.

3. What mechanisms determine the number of cells that belong to stage 5?

The mechanisms that determine the number of cells in stage 5 are as follows:

- Differentiation from stage 4 to stage 5: stage 4 cells differentiate into stage 5 through the activity of myogenin activity. Strain-related signaling molecules further enhance myogenin's ability to bind to the MHC promoter, increasing MHC expression and forming more mature muscle cells.
- Cell death in stage 5: some stage 5 cells go into apoptosis, removing them from the stage 5 population.

Because stage 5 is the most mature in the model, there is no further differentiation out of stage 5, and these two processes (differentiation from stage 4 and cell death) fully determine the number of cells in stage 5.

4. What biological mechanism is behind the effect of strain on myogenic differentiation?

The effect of strain on myogenic differentiation is primarily mediated by a strain-induced molecule S, which represents mechanotransduction effects such as integrin-mediated FAK activation and involvement of the Rho pathway.

When strain is applied, it promotes the production of S, which modulates the activity of transcription factors such as myogenin and MyoD. This modulation enhances the binding of MyoD to the myogenin promoter, leading to increased myogenin expression and then MHC expression, promoting the progression of myogenic differentiation.

5. What are the differences between the mechanisms of cell differentiation in stages 2 and 5?
- **Stage 2:** is an early, proliferative stage where cells with expressed Desmin (DES+) are generated from the previous stage 1. They can both self-renew and differentiate further into stage 3
  - **Stage 5:** is the terminal stage (MHC+); cells arise here only through direct differentiation from stage 4 (driven by strain-enhanced myogenic activity) and do not proliferate, cell death being the only loss mechanism.

6. What are the differences between the mechanisms of cell differentiation in stages 3 and 5?

Stage 3 (MyoD+)

- Intermediate stage
- Can gain new cells through division of stage 2 cells and cells can also self-renew.
- Stage 3 cells express MyoD. Their next differentiation step involves myogenin, leading them into stage 4.

Stage 5 (MHC+)

- Terminal stage— Stage 5 gains cells exclusively via direct differentiation from stage 4. There is no self-renewal or outflux from stage 5—only cell death.
- No further differentiation: stage 5 cells express MHC, the biomarker of mature muscle cells. No further transcription factor drives them into another stage.