The Role of the Super Elongation Complex (SEC) in Epidermal Progenitor Maintenance

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Abstract

The basal layer of human epidermis is composed of progenitor cells that both self-renew and differentiate to form stratified epidermal tissue; this process requires strict, dynamic regulation of gene expression. The Super Elongation Complex (SEC) regulates gene expression at the level of transcription elongation by releasing RNA Polymerase II from its paused state. Dysregulation of the SEC has been identified in leukemia and fragile X syndrome, but how the SEC functions in somatic tissue homeostasis has not been characterized. To better understand the role of the SEC, we performed a knockdown and an evolutionary analysis of the SEC's mutually exclusive scaffolding subunits, AFF1 and AFF4. Our data revealed a difference in function between AFF1 and AFF4. Knockdown of AFF1, but not AFF4, resulted in increased expression of epidermal differentiation genes, suggesting that AFF1-SEC may function as a transcription inhibitor to promote progenitor maintenance. The evolutionary analysis revealed that *Homo sapiens* AFF1, but not AFF4, has a region of homology to both the Atrophin and Herpes ICP4 protein domains, both of which possess inhibitory activity. Given that *Drosophila melanogaster* has only one AFF homologue, this analysis suggests that AFF proteins evolved novel functions to promote more complex patterns of gene expression in higher-order species. The observed differences between AFF1 and AFF4 suggest a mechanism through which the SEC selectively utilizes AFF1 to suppress expression of differentiation genes in order to maintain cells in the progenitor state.

A Model of the SEC in *Homo sapiens* **B** Model of the SEC in *Drosophila melanogaster*

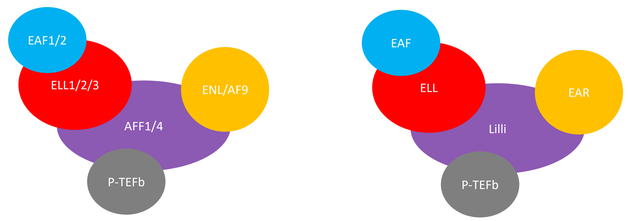
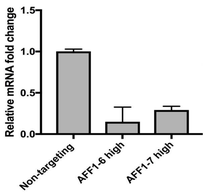
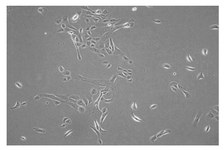


Figure 1: The SEC is composed of different protein homologues in *Homo sapiens* and *Drosophila melanogaster* **A.** The *Homo sapiens* SEC has multiple variants of several of its component proteins **B.** The *Drosophila melanogaster* SEC has only one known variant of each of its component proteins

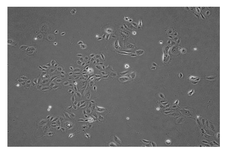
A Knockdown efficiency of shRNAs AFF1-6 and AFF1-7



B Cells knocked down with shRNA AFF1-6 show reduced ability to proliferate



C Cells knocked down with shRNA AFF1-7 show reduced ability to proliferate



D Control cells treated with non-targeting shRNA

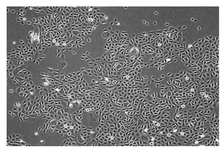
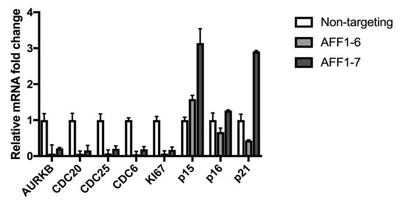
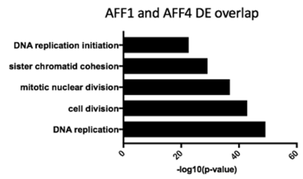


Figure 2: Knockdown of AFF1 with shRNAs AFF1-6 and AFF1-7 was successful **A.** qPCR of the expression level of AFF1 in samples treated with non-targeting shRNA, and shRNAs AFF1-6 and AFF1-7 **B.** Image of cells knocked down with shRNA AFF1-6 **C.** Image of cells knocked down with AFF1-7 shRNA **D.** Image of cells knocked down with non-targeting shRNA

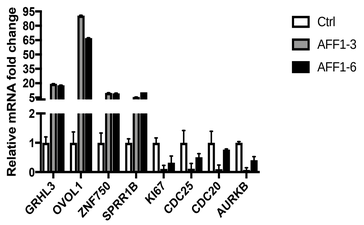
A AFF1 knockdown downregulates cell-cycle activating genes



B AFF1 and AFF4 activate transcription of cell-cycle activating genes



C AFF1 knockdown upregulates keratinocyte differentiation genes



D AFF1, but not AFF4, inhibits transcription of differentiation genes

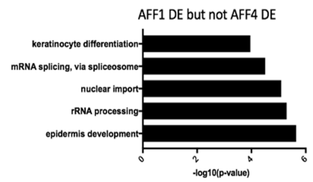
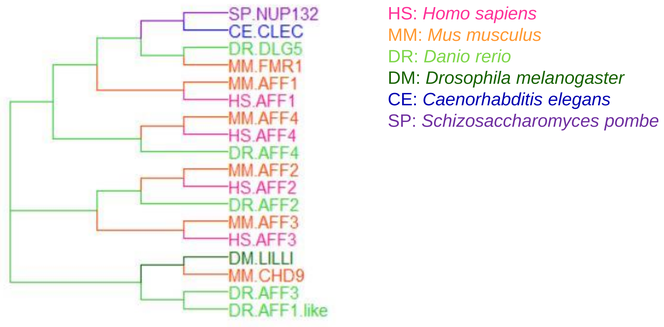


Figure 3: While AFF1 and AFF4 are both transcription activators, AFF1 may have a novel function as a transcription inhibitor **A.** qPCR of changes in gene expression of cell-cycle genes after AFF1 knockdown **B.** GO term analysis of genes targeted by both AFF1 and AFF4 **C.** qPCR of changes in gene expression of keratinocyte differentiation markers after AFF1 knockdown **D.** GO term analysis of genes targeted by only AFF1, not AFF4.

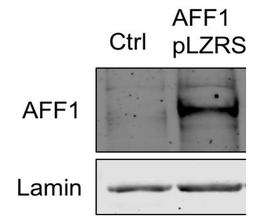
A *Homo Sapiens* AFF1 is more closely related to AFF homologues in less-evolved species than is AFF4



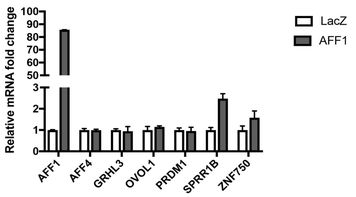
B AFF1 has homology to known inhibitory domains while AFF4 does not

Figure 4: Evolutionary analysis of the AFF protein family reveals potential differences between AFF1 and AFF4 **A.** Phylogenetic tree constructed with Cobalt and ggtree show proposed evolutionary relationship of AFF proteins **B.** Depiction of the AFF proteins of three species with conserved domains, found from Batch, shown in color and numbered by the amino acids at which they occur.

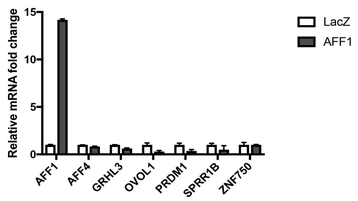
A FHH-Tagged AFF1 was successfully expressed at Day 0 of Differentiation



B Day 0: Overexpression of AFF1 did not affect differentiation markers



C Day 2: Overexpression of AFF1 downregulated differentiation markers



D Day 4: Overexpression of AFF1 downregulated differentiation markers

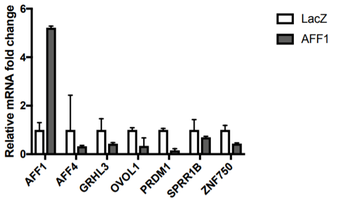


Figure 5: Overexpression of AFF1 downregulates keratinocyte differentiation markers **A.** Western Blot of undifferentiated cells treated with FHH-tagged AFF1 in pLZRS vector and probed for the HA tag **B.** qPCR of changes in gene expression of differentiation markers after AFF1 overexpression in undifferentiated keratinocytes (Day 0) **C.** qPCR of changes in gene expression of differentiation markers after AFF1 overexpression in keratinocytes induced to differentiate (Day 2) **D.** qPCR of changes in gene expression of differentiation markers after AFF1 overexpression in keratinocytes induced to differentiate (Day 4)

Conclusions

- The SEC activates transcription of cell-cycle activator genes
- The SEC inhibits transcription of keratinocyte differentiation markers when in complex with AFF1, but not when in complex with AFF4
- AFF1, but not AFF4, has a region of homology to the Atrophin and Herpes ICP4 domains with known inhibitory functions
  - This homology is conserved from the AFF proteins in *Danio rerio*
- Overexpression of AFF1 inhibits keratinocyte differentiation

Future Directions

1) Verify that AFF4 does not inhibit expression of differentiation markers
-Repeat differentiation assay, overexpressing AFF1 in one sample and AFF4 in another sample

2) Is AFF1's region of homology to the conserved domains necessary and sufficient for it to function as an inhibitor?

a) Knockdown and rescue
-Knockdown AFF1 using shRNA AFF1-6 and rescue with full length Site Directed Mutagenesis AFF1 construct
-Knockdown AFF1 and rescue with the region of homology
-Knockdown AFF1 and rescue with an AFF1 construct with the region of homology deleted

b) Differentiation assay
-Repeat differentiation assay, overexpressing only the region of homology
-Repeat differentiation assay, overexpressing with AFF1 construct with region of homology deleted

The study resulting in this publication presentation was assisted by a grant from the WCAS Undergraduate Research Grant Program which is administered by Northwestern University's Weinberg College of Arts and Sciences. However, the conclusions, opinions, and other statements in this presentation are the author's and not necessarily those of the sponsoring institution.