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Title	Mechanical role of spear-shaped collagen crystals in the caudal fin formation of zebrafish
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Citation	大阪大学, 2022, 博士論文
Version Type	VoR
URL	https://doi.org/10.18910/88167
rights	
Note	

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論　文　内　容　の　要　旨

氏　名　（　中川　日々紀　）	
論文題名	Mechanical role of spear-shaped collagen crystals in the caudal fin formation of zebrafish （ゼブラフィッシュの尾ヒレ形成において棒状コラーゲン結晶体が果たす役割の解明）

論文内容の要旨

Zebrafish show a V-shaped caudal fin, with 17 to 18 fin-bones (fin-rays) extending from proximal to distal. Each fin-ray is composed of dozens of repeating segments, which are added distally to elongate the fin-ray and branch in the distal part of fins. However, the fin-rays only extend to the front of the leading edge of the fin growth, beyond which there are no fin-rays, but spear-shaped collagen crystals called actinotrichia. Although previous studies have shown that actinotrichia are essential for fin development and regeneration, their specific role in fin formation has not been clarified. In this study, I aimed to elucidate the role of the orderly arrangement of actinotrichia at the distal tip of the fin in zebrafish fin formation.

Firstly, I identified the *collagen9a1c* (*col9a1c*) gene that was presumed to be involved in the actinotrichia arrangement to generate mutants with the disturbed alignment of actinotrichia in the fin. I then generated *co9a1c*-knockout zebrafish using the CRISPR/Cas9 system and observed their phenotypes during growth. As a result, I found that the knockout fins shrank in the dorsoventral axis as the fish grew. The abnormality in the fins became more pronounced around three weeks of age, when the fin-rays began to form.

Next, I performed a quantitative analysis of the fin and fin-rays phenotypes in six categories to obtain a more detailed understanding of them. The phenotypes in which significant differences were observed in the *col9a1c*-knockout mutant compared with the wild-type were the following four: decrease in the fin length in the dorsoventral axis, decrease in the segment length of fin- rays, decrease the number of fin-rays at the distal part of the fin, and decrease in the branching frequency of fin-rays. This indicates that the deletion of *col9a1c* significantly affects the fin and fin-ray phenotypes.

I then observed the arrangement of actinotrichia using the transgenic zebrafish in which actinotrichia were visualized. I found that actinotrichia were initially produced normally and aligned correctly in the *col9a1c*-knockout mutant, but as the fish grew, the number of actinotrichia decreased, and their arrangement became disordered, just as the abnormalities in the fin shape appeared with growth. Moreover, actinotrichia were usually aligned without gaps at the distal fin tip to form a thin two-dimensional plane. In contrast, actinotrichia were sparsely arranged in the knockout mutant, suggesting that cells adjacent to actinotrichia lost mechanical support at the fin tip.

Finally, to investigate the physical impact of the loss of orderly actinotrichia arrangement on the surrounding cells, I observed the shape of the two epidermal cells— peridermal cells and basal keratinocytes—at the fin tip and thickness of the tissue. Similar to the phenotype of the fin shape and the actinotrichia arrangement, there were no significant changes in the cells shape or the tissue thickness in the *col9a1c*-knockout mutant at 5-day-old, but markedly differences in them appeared at 3-week-old fish. The size of the superficial cell was reduced by about half, and that of the basal keratinocytes was decreased by about one-third. In addition, the basal keratinocytes, which are normally thinly spread, were spherical in the knockout mutant. Furthermore, the thickness of the tissue was doubled toward the distal fin tip.

These results suggest that the orderly arrangement of the actinotrichia at the fin tip provides a physical scaffold for epidermal cells and tissue to spread thinly and for the fin to grow in the dorsoventral direction.

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論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

中川氏は、ゼブラフィッシュヒレの形態形成のカギとなるコラーゲン結晶体であるアクチノトリキアに関する分子遺伝学的研究を行い、画期的な発見をした。アクチノトリキアが無くなると、ヒレそのものができなくなってしまうことから、アクチノトリキアがヒレ形成に重要であることが解っていたが、その詳しい役割等は、ほとんど不明であった。中川氏は、アクチノトリキアの正確な配列自体が、ヒレ骨形成のベースとなると予想し、アクチノトリキアの配列異常を持つ変異体を*col9a1c*遺伝子のKOで作り出した。さらにアクチノトリキアの配列が、ヒレ先端に平面状の足場を作り、ヒレの細胞はその場を利用して、ヒレを作ることが解った。さらに*col9a1c*遺伝子産物は、アクチノトリキアを整列させる間葉系細胞のマーカーとなり、ヒレ形成に寄与することも見出している。これらの発見は、これまで形態形成研究の中心であった胚ではなく、成体における形の原理を明らかにするものとして画期的であり、博士号を授与するに足ると判断した。

近藤滋