INTRODUCTION

In many vertebrate species, including mouse and human, the flexor muscles that govern motor control of the hand (autopod) are composed of two distinct functional groups: (1) extrinsic muscles that reside exclusively within the forearm (zeugopod) and connect to the long tendons of the autopod and (2) intrinsic muscles that are localized completely within the autopod with their attaching tendons [1]. The muscles and tendons of the first type include the flexor digitorum sublumis (FDS). In the current study, we traced the remarkable development of the FDS tendons and muscles and showed that the sublimis muscles first form as differentiated myofibers in the autopod before moving to their final location in the zeugopod. This finding is exceptional as there are no reports in the literature of muscles moving as multi-nucleated, differentiated fibers during development [2]. We also demonstrated that FDS tendon and muscle development is tightly coupled; the formation of tendon is dependent on active translocation of its muscles and muscle translocation in turn, is dependent on tendon. The development of the sublimis tendon and muscles has clinical significance as there are several pathologies involving ectopic FDS muscles within the wrist or attached to tendons [3], giving rise to hand and wrist pain. Understanding the genetic basis of these developmental processes may inform clinical treatments.

MATERIALS AND METHODS

Existing mouse lines used in this study were previously described: ScxGFP [4], HB9Cre;Ist2DTA [5], mdg [6], ScxNull [7]. Whole mount myosin staining, in situ hybridization, and immunostaining were performed as previously described [7].

RESULTS

While almost all of the tendons of the forelimb, including the FDP tendons, were fully formed at E14.5, the FDS tendons were not completely formed until E16.5 [1]. At E14.5, only a segment at the metacarpophalangeal joint was present; the digit and metacarpal segments were entirely absent. Instead, three unidentified muscles were observed in place of the metacarpal tendons (Fig 1). At this stage, these muscles were intrinsic (restricted to the autopod) and did not extend past the wrist. To determine the identity of these unexpected muscles, we followed their fate through E14.5-E16.5. Whole mount myosin staining showed that during these stages, the muscles elongated and translocated into the zeugopod as differentiated muscles (Fig 2A), with the FDS tendons forming in their wake (Fig 2B). By E16.5, the FDS tendons were fully formed, extending from the autopod to the zeugopod, with the FDS muscles exclusively localized within the zeugopod.

At E14.5, only a small segment of the FDS tendon was present at the metacarpophalangeal joint [1]. As this structure was formed before the initiation of muscle translocation, we wondered whether interaction between these tissues was important for muscle movement. We therefore examined muscle translocation in the ScxNull mutant, in which the metacarpal FDS tendon segments do not form [7]. Myosin staining showed a failure of FDS muscle translocation at E16.5 in the ScxNull (Fig 3), suggesting that tendon is required for muscle movement.

One interesting structural feature of the FDS muscles at E14.5 was the presence of motoneurons within each muscle (not shown). Their intriguing presence prompted us to determine whether muscle activity was required for translocation. We examined the muscles and tendons of two mutants lacking muscle contraction: HB9Cre;Ist2DTA and mdg. While the muscles and tendons of the HB9Cre;Ist2DTA mice appeared normal (albeit smaller) at E16.5 (Fig 4), the metacarpal tendons of the mdg mice failed to form; instead the FDS muscles remained localized within the autopod, fusing and crossing the wrist as a single muscle (Fig 4).

DISCUSSION

The conflicting phenotypes between HB9Cre;Ist2DTA and mdg were unexpected given that muscle contraction was impaired in both mutants. The mechanisms by which muscle contraction was disrupted in the two mouse strains were distinct however. In the HB9Cre;Ist2DTA, the lack of muscle contraction was caused by loss of motoneurons. In mdg, the lack of excitation-contraction coupling was due to a loss-of-function mutation in calcium signaling [8]. Therefore, the sublimis phenotype in mdg may be due to a failure in calcium signal propagation within these muscles (independent of muscle contraction). While the presence of motoneurons within these muscles was suggestive, results from the HB9Cre;Ist2DTA mouse indicate that muscle contraction is not a requirement for FDS muscle translocation. The role (if any) of these neurons therefore remains to be elucidated. In the absence of tendon, muscle translocation did not occur; however, whether the effect of tendon on muscle movement was direct or indirect is unclear and will be the focus of future studies.

ACKNOWLEDGEMENTS

This work was supported by NIH grant R01AR055973 from NIAMS.

REFERENCES