Grafting experiments from Wenger and Wenger have shown that the identity of spinal cord cells (albeit posterior sections to cervical levels) is controlled by signaling centers along both the anterior to posterior and dorsal to ventral axes of the chick neural tube (Wenger 1950, Wenger 1951). Another study by the Hamburger lab found that brachial and lumbar grafts have regional spinal cord identity (Narayanan and Hamburger 1971).

At stage 8 of chick neural tube development, neurulation and neural tube formation has completed (Bellairs and Osmond 2005). It is from this developmental timepoint that we begin our experiment.

A) Upon an Anterior-Posterior (A-P) reversal graft, I predict that the polarity of the spinal cord will be reversed such that Cervical level 6 is now anterior and Cervical level 2 is posterior (**retain ectopic polarity**). They will retain their same target axial muscles. This is because A-P patterning has already begun at the stage of the neural plate (Lumsden and Krumlauf 1996). The demonstration of retained neural identity upon grafting at this timepoint has been demonstrated elegantly in the hindbrain with rhombomere (r) transplants. Simon et al. grafted r-4 to r-2 position and observed the characteristic facial motor neurons and contralateral vestibulo-acoustic efferent neurons of r4 in the grafted position of r-2 (Simon, Hornbruch et al. 1995). Regarding cell type identity, projections and circuit organization, I predict that these properties will assume the ectopic properties of the grafted tissue. This will result in abnormal wiring of cervical axial muscles such as the neck muscles

Upon a Dorsal-Ventral (D-V) inversion graft, I predict that the polarity of the spinal cord will be normal (**retain endogenous polarity**). Again, taking lessons from Simon et al., they found a re-patterning along the D-V axis of the hindbrain upon inverted graft of r-4, suggesting that cells are still labile with respect to D-V polarizing signals from notochord/floor plate (Simon, Hornbruch et al. 1995). The notochord and floorplate have the ability to repattern when ectopically transplanted (Yamada, Placzek et al. 1991). Furthermore, the notochord derived Shh signaling is constant along the rostrocaudal axis (Yamada, Placzek et al. 1991), suggesting that these signals will be retained upon graft.

B) Concerning the stage of the graft being earlier (stage 4) or later (stage 17) depends on the pluripotency of cells. At stage 4, epiblast cells have yet to become neural progenitors. H&H staging defines that the primitive streak has reached its maximum length and the neural tube has yet to close (Hamburger and Hamilton 1951, Bellairs and Osmond 2005). Therefore, I predict that grafting at this stage would still permit **normal** neural tube patterning and differentiation.

In contrast, at stage 17 the neural tube has closed, organizer signals have induced A-P patterning and the notochord/somite structures are present which have initiated D-V patterning (Hamburger and Hamilton 1951, Bellairs and Osmond 2005). Therefore, I predict that either grafts will retain their ectopic fates and thus the chick spinal cord will be abnormal. Additionally, by stage 17, Hoxc5 (an intrinsic transcription factor) was detected throughout the cervical spinal cord which restricts this region to a defined spinal cord fate (Liu, Laufer et al. 2001).

C) I’ve provided evidence from previous transplant/graft experiments which have guided my predictions here, but the question remains, how is induction or re-patterning achieved?

Key signaling pathways, both extrinsic and intrinsic have been implicated. Beginning with neural induction, the inhibition of BMP signaling can be induced by three key proteins: follistatin, noggin and chordin, all expressed by the organizer cells, or dorsal lip of the gastrula (Lumsden and Krumlauf 1996, Jessell 2000). Retinoic acid (RA) also has a role in patterning the spinal cord, where it regulates expression of intrinsic factors like Hox genes associated with rostral cervical levels (Liu, Laufer et al. 2001).

Other extrinsic players involved in D-V patterning are BMPs (dorsalizing signal emanating from roof plate cells) and Shh (ventralizing signal from notochord) (Tanabe and Jessell 1996, Patten and Placzek 2002). Wnt proteins (1 and 3) is a signal that induces differentiation of the dorsal spinal cord (Lee and Jessell 1999). The FGF family of proteins is another signal that can induce neurulation (Tanabe and Jessell 1996).

Lastly, a role of spontaneous network activity may contribute to spinal cord development (Kerschensteiner 2013). For example, O’Donovan et al. found spontaneous activity of hindlimb muscles recorded in ovo as well as in vitro (O'Donovan, Chub et al. 1998). Episodic network activity was also found in the developing mouse spinal cord (Hanson and Landmesser 2003). Whether they have a role in spinal cord circuit maturation is poorly understood…

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