Joseph A. Holden
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The Zebrafish

Atlas of Macroscopic and Microscopic Anatomy

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The zebrafish (*Danio rerio*) is a valuable and common model for researchers working in the fields of genetics, oncology, and developmental sciences. This full-color atlas will aid experimental design and interpretation in these areas by providing a fundamental understanding of zebrafish anatomy.

Over 150 photomicrographs are included and can be used for direct comparison with histological slides, allowing quick and accurate identification of the anatomic structures of interest. Hematoxylin and eosin (H&E) stained longitudinal and transverse sections demonstrate gross anatomic relationships and illustrate the microscopic anatomy of major organs. Unlike much of the current literature, this book is focused exclusively on the zebrafish, eliminating the need for researchers to exclude structures that are only found in other fishes.

Joseph A. Holden was a member of the Department of Pathology at the University of Utah School of Medicine. His most recent interests included the development of a zebrafish model for c-kit and malignant melanoma as well as a zebrafish model for BRAF and carcinoma of the thyroid.

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Jennifer L. Matthews works at the Zebrafish International Resource Center at the University of Oregon. She has extensive experience working with these animals and is an active member of the zebrafish community.

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Preface

The present atlas is designed to aid basic and translational scientists who require a fundamental understanding of zebrafish macro- and micro-anatomy. Many investigators with interests in the molecular features of oncogenesis or molecular genetics and organ development have found zebrafish to be a valuable animal model for their studies. However, these investigators often lack basic training in the fundamentals of fish histology and anatomy. This book is intended to address that gap.

The present atlas makes use of H&E stained longitudinal and cross sections to demonstrate the macro- and micro-anatomy of the zebrafish. Unlike many other atlases, all the photographs in the present book are obtained from zebrafish. This is important because many species of bony fishes show considerable anatomic variation. While some bony fishes possess a tongue and a true stomach, these are absent in zebrafish resulting in significant anatomic differences. The text concentrates on elucidating the actual microscopic appearance of zebrafish. An initial chapter uses longitudinal and cross sections photographed at low or no magnification to illustrate the relationships of zebrafish anatomy. Later chapters follow an organ systems approach in which the histology at both low and high power is addressed in detail. It is hoped that this combined approach will allow investigators to quickly and accurately identify specific organs and tissues involved by neoplasms or developmental abnormalities induced by molecular genetic changes. To this end, the text accompanying the photomicrographs has been made relatively brief while a large number of photomicrographs have been included. The photomicrographs have been generously labeled for easy identification of structures within the tissue sections. Correlation of photomicrographs present in the organ-based chapters with those present in the orientation chapter should allow rapid identification of tissue structures observed in study preparations.

Interpretation of histologic slides requires the recognition of forms, organization, and location from where the tissue section was taken within the fish's body. Although color is less important in the identification of tissue type than structure, the present atlas has been photographed entirely in color to facilitate rapid identification of tissue structures. Color photographs facilitate comparison of the findings on glass slides with the photomicrographs within the atlas. It should be borne in mind that specific color hues may vary between glass slides produced by different laboratories of identical tissues and between a given laboratory and the photomicrographs present in the atlas. However, it should be remembered that differences in color between tissues within a given slide are more important than modest variations between the glass slide and the histology atlas.

The atlas is intended to be used in the laboratory as a quick reference for identification of tissues seen in H&E stained preparations of zebrafish specimens. The atlas uses photomicrographs rather than composite illustrations because the former present more practical information allowing direct comparison between illustrations within the atlas and the slide under study.

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CHAPTER ONE
Introduction

Utility of zebrafish as an animal model for study of oncogenesis and developmental defects

The development of animal models for a variety of neoplasms has greatly facilitated our understanding of oncogenesis and the relationship between somatic and germ line mutations with tumor growth and development. While mammalian models for developmental abnormalities and neoplasia would appear most appropriate, they are often hindered by issues of cost, latency to expression of phenotype, and animal care issues. Hence, non-mammalian but vertebrate organisms have a number of advantages. The zebrafish (*Danio rerio*) has emerged as a useful model system for the study of cancer biology because it has a reduced latency to expression of phenotype, a relatively low cost, a susceptibility to many tractable techniques for analysis of gene function, and the species amenability to oncogenic and chemical modifiers. In addition, early zebrafish embryos are optically clear, allowing observation of tumor development and organogenesis. This allows in vivo examination of cell and tissue behavior. A number of zebrafish models of neoplasia have been developed for both inactivating mutations[1] and for the expression of human oncogenes including C-MYC, BRAF, and N-ras, which are known to be associated with a variety of human neoplasms[2–4].

The majority of living fishes including the zebrafish are members of the division *Teleostei*. This division represents the most advanced of the living bony fishes accounting for 96% of all fish species. Teleosts occur in both fresh and marine water habitats. The order *Cypriniformes* includes the zebrafish and other popular aquarium fishes including the goldfish and koi. Because these fishes are relatively easy to raise and the maintenance of colonies of these fishes is straightforward, members of this order have become popular for hobbyists and researchers alike. The zebrafish is the standard research animal for developmental genetics as well as being a popular species for the aquarium enthusiast[5].

Despite the utility of the zebrafish model for studying neoplasia, relatively little information has been published concerning the anatomy, histology, and histopathology of the zebrafish. While significant histologic and anatomic overlap exists between certain organs (e.g. pancreas) in both mammals and zebrafish[6], the appearance of other organs and tissues varies greatly in distribution or appearance between mammals and fishes. The present text is designed to facilitate identification of organs both unique to the zebrafish and those common to both fish and mammals. For orientation purposes, low power photographs of cross sections and longitudinal sections of zebrafish are provided as an initial step toward organ identification within the zebrafish. Subsequent chapters review zebrafish histology at medium to high power. These latter chapters follow an organ system approach but are cross-referenced with the earlier anatomically based photomicrographs and descriptions. The atlas is designed for use in the laboratory when examining gross sections of the zebrafish as well as H&E stained microscopic sections.

Fixation and histologic methods for microscopy of zebrafish

In all cases, the photomicrographs are taken of formalin-fixed and paraffin-embedded fish tissues. This presentation minimizes tissue distortion and allows accurate sectioning of the specimens for microscopic evaluation. While other staining techniques exist, the H&E staining method optimizes evaluation of microanatomy with excellent preservation of nuclear and cytoplasmic detail. In addition, extracellular substances frequently display characteristic tinctorial reactions with the H&E technique, which are not replicated by other staining techniques such as geimsa, crystal violet, or methylene blue. While the photomicrographs were obtained from formalin-fixed paraffin-embedded material, their appearance will be similar to that of H&E stained frozen section (cryostat) specimens. Hematoxylin and eosin is the stain most commonly used in preparing routine histology/histopathology specimens. Hematoxylin is predominately a nuclear stain but does have some affinity for cytoplasmic components including ribosome-rich tissues and extracellular materials such as the matrix of cartilage. The eosin dye stains most cytoplasmic components including intermediate filaments and extracellular fibers. The interplay of these two basic stains produces the information-rich tapestry of the routine H&E section.

Optimal fixation and tissue processing is achieved with 10% neutral buffered formalin or a number of fixative mixtures (Dietrich's, Davidson's, Bouin's, or Lillie's fixatives[7]) followed by serial dehydration through graded ethanols to xylene with final impregnation with paraffin wax and the preparation of paraffin blocks. Because zebrafish are small, the fish can be fixed whole. For optimum results, fish must be alive and euthanized just prior to fixation. Autolysis occurs rapidly once a fish dies. The volume of fixative should be 10 to 20 times the volume of the specimen. Cutting a small opening in the abdominal wall will aid fixative penetration and preservation of visceral tissues. Fish should be placed in a container in a horizontal position with gentle agitation (e.g. laboratory rocker) for the first 24 hours of fixation. This will ensure optimal fixation and prevent artificial bending of the body axis. Following fixation, specimens should be cut at a thickness of 3 to 4 mm to insure adequate processing through paraffin. In most instances, 5 μm sections are optimal for light microscopy and are cut on a standard microtome. Moore *et al.*[8] have described methods for the fixation and decalcification of zebrafish. For some special techniques, frozen-section processing is optimal. In these cases, tissue blocks of approximately 4 mm are prepared. These are mounted on cryostat chucks using an embedding medium such as optimal cutting temperature (OCT) medium. As with formalin-fixed paraffin-embedded tissues, 5 μm sections are cut and kept frozen for preparation of the desired special technique.

A variety of staining techniques exist in addition to the H&E method (Table 1.1). These stains highlight particular cellular and extracellular substances including collagen, mucopolysaccharides, reticulum, smooth muscle, glycogen, mucins, and a variety of lipid substances. Details of these staining methods can be found in specialty texts[9–11].

Immunohistochemistry allows specific recognition of a number of protein components. While some commercially available antibodies will react specifically with the desired antigen in zebrafish, others are specifically raised for mammalian proteins and may fail to react or react non-specifically in zebrafish specimens.

Table 1.1 Common histochemical stains and their uses.

Alician blue	acid mucopolysaccharides (mucins)
Bielchowsky stain	nerve fibers
Brown and Brenn (tissue Gram stain)	Gram + and – bacteria
Colloidal iron	acid mucopolysaccharides
Congo red	amyloid
EVG stain	elastin
Holzer's stain	glial fibers
Grocott's methenamine silver stain	fungus
Mallory's iron stain	iron
Jones' silver stain	basement membrane
Mayer's mucicarmine stain	mucins
Periodic acid–Schiff (PAS)	glycogen + mucopolysaccharides
Gordon and Sweet's reticulin stain	reticulin fibers
Masson's trichrome stain	collagen and smooth muscle

References

1. **Cheng R, Ford B L, O'Neal P E,** *et al.* Zebrafish (*Danio rerio*) p53 tumor suppressor gene: cDNA sequence and expression during embryogenesis. *Mol Mar Biol Biotechnol* 1997;**6**(2):88–97.
2. **Patton E E, Widlund H R, Kutok J L,** *et al.* BRAF mutations are sufficient to promote nevi formation and cooperate with p53 in the genesis of melanoma. *Curr Biol* 2005;**15**(3):249–54.
3. **Langenau D M, Traver D, Ferrando A A,** *et al.* Myc-induced T cell leukemia in transgenic zebrafish. *Science* 2003;**299**(5608):887–90.
4. **Stern H M & Zon L I.** Cancer genetics and drug discovery in the zebrafish. *Nat Rev Cancer* 2003;**3**(7):533–9.
5. **Spitsbergen J M & Kent M L.** The state of the art of the zebrafish model for toxicology and toxicologic pathology research – advantages and current limitations. *Toxicol Pathol* 2003;**31** Suppl:62–87.
6. **Davison J M, Woo Park S, Rhee J M & Leach S D.** Characterization of Kras-mediated pancreatic tumorigenesis in zebrafish. *Methods Enzymol* 2008;**438**:391–417.
7. **Fournie J W, Krol R M & Hawkins W.** Fixation of fish tissues. In Ostrander G K (ed.) *The Laboratory Fish.* (London, England: Academic Press, 2000), pp. 569–77.
8. **Moore J L, Aros M, Steudel K G & Cheng K C.** Fixation and decalcification of adult zebrafish for histological, immunocytochemical, and genotypic analysis. *Biotechniques* 2002;**32**(2):296–8.
9. **Carson F L & Hladik C.** *Histology: A Self-instructional Text*, 3rd edn. (Chicago, IL: American Society for Clinical Pathology Press, 2009).
10. **Bancroft J D & Gamble M.** *Theory and Practice of Histological Techniques*, 6th edn. (Philadelphia, PA: Churchill Livingstone Press, 2007).
11. **Schilling T F.** The morphology of larval and adult zebrafish. In Nüsslein-Volhard C & Dahm R (eds.) *Zebrafish.* (Oxford: Oxford University Press, 2002) pp. 68–90.

CHAPTER TWO
Cross section and longitudinal section atlas

The following two sections catalog cross sections and longitudinal sections of adult male and female zebrafish. The sections presented are not serial sections but are representative sections presented sequentially to illustrate important anatomical relationships. These sections serve as references to more detailed discussions of specific organs in subsequent chapters. The cross sections follow organ location and relationships through the zebrafish in a rostral to caudal direction. Because the sections are not precisely sequential, the size of a given organ may vary greatly from one illustrated section to the next.

Similarly, the longitudinal sections are taken from right to left. The extreme peripheral areas of the fish are not illustrated because little significant anatomic information would be supplied as most of these far peripheral sections are composed of striated muscle, bone, cartilage, and integument. As with the cross sections, both male and female fish are illustrated.

Cross section atlas

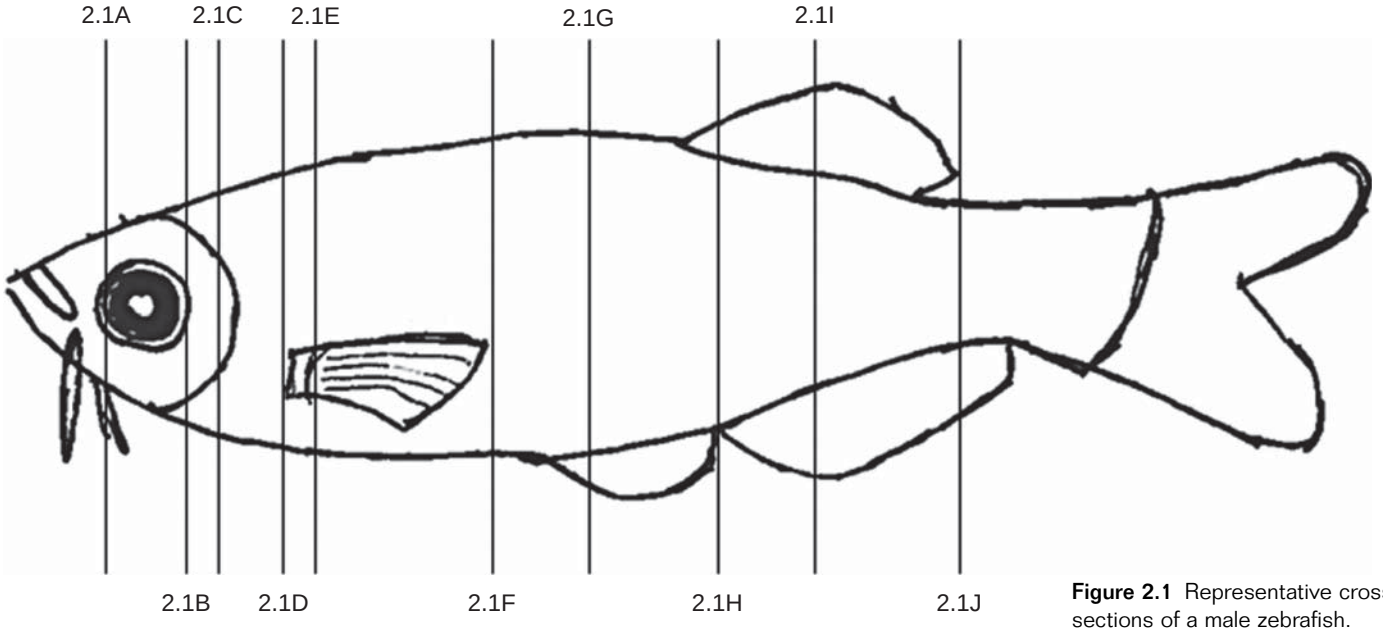

Figure 2.1 Representative cross sections of a male zebrafish. Figures 2.1A to 2.1J show relative positions of cross sections.

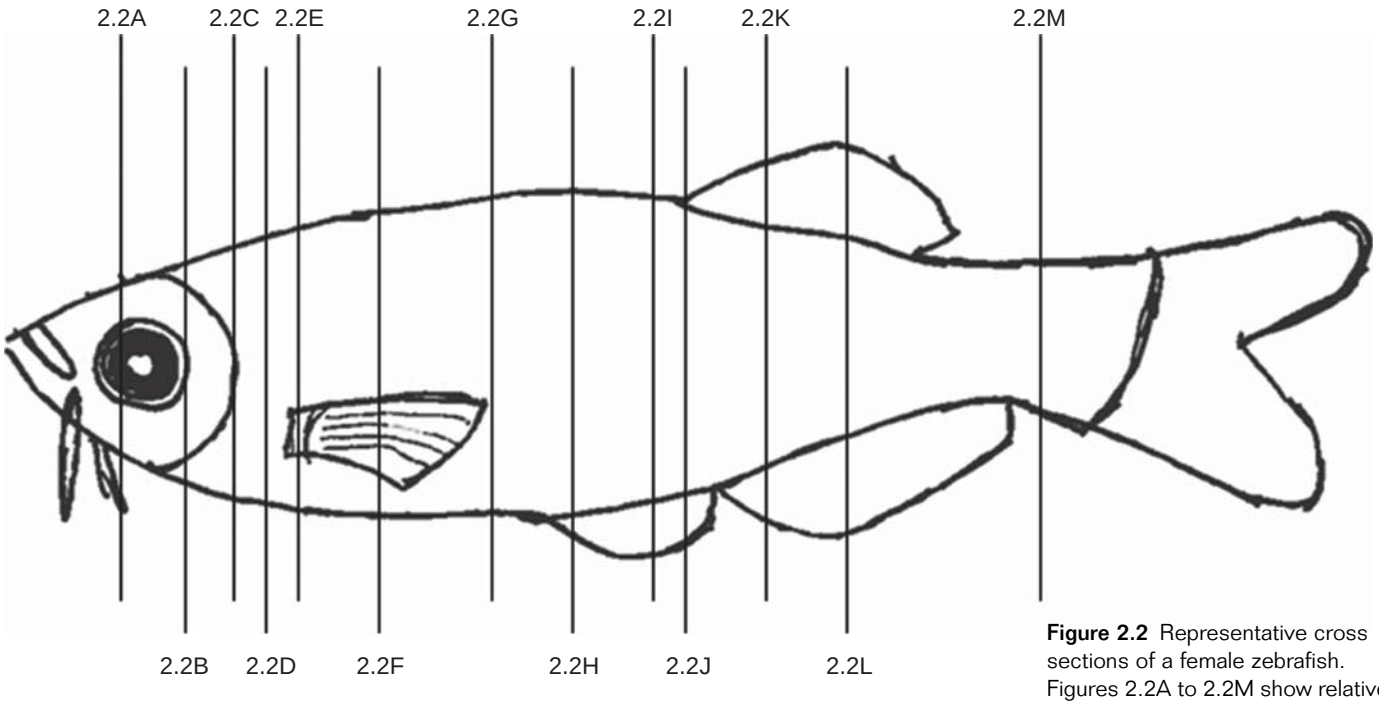

Figure 2.2 Representative cross sections of a female zebrafish. Figures 2.2A to 2.2M show relative positions of cross sections.

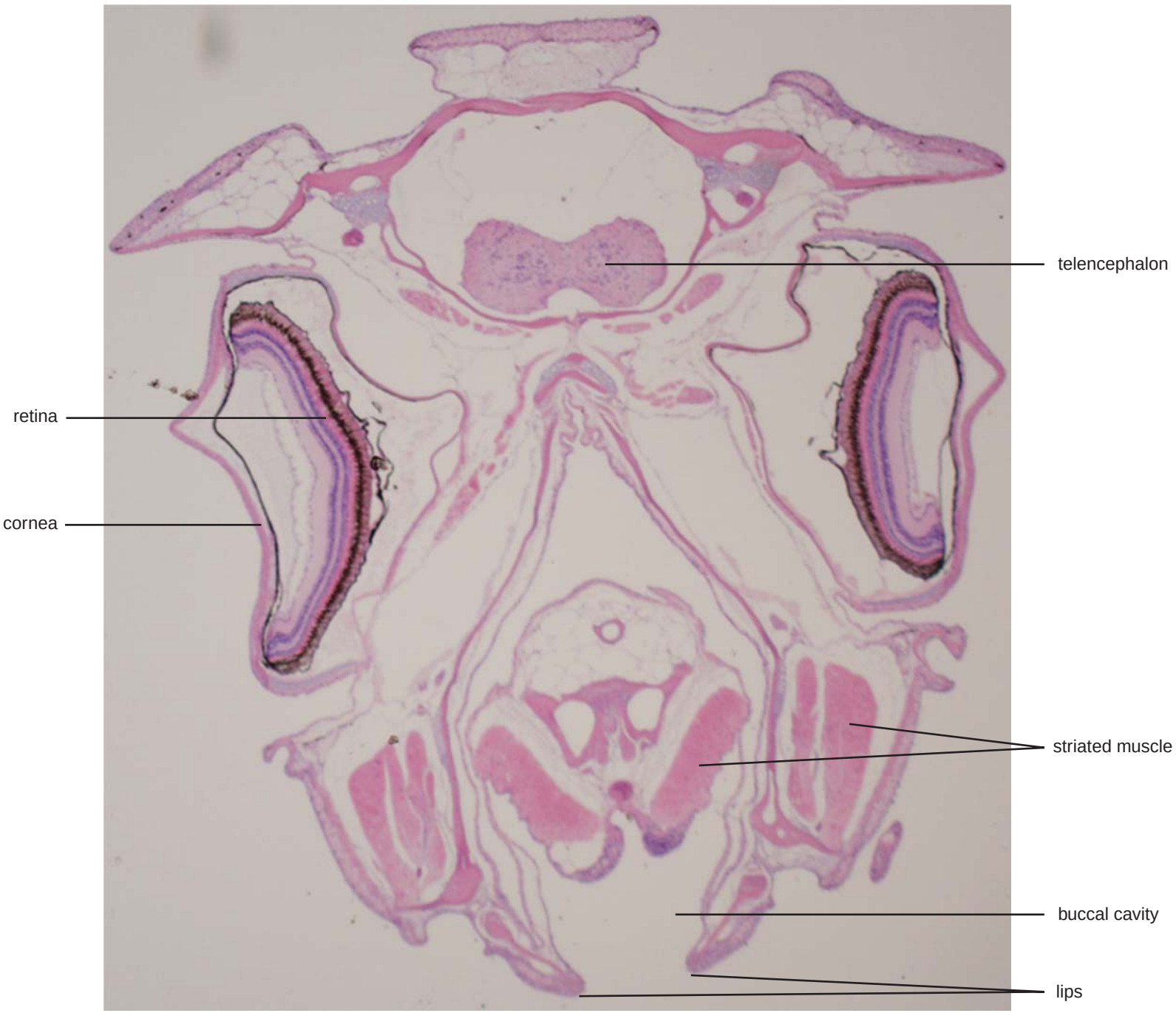
telencephalon
retina
cornea
striated muscle
buccal cavity
lips

Figure 2.1A