

**The amphibian invitrome: Past, present, and future contributions to our understanding of
amphibian immunity**

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Abstract

Many amphibian populations are declining worldwide, and infectious diseases are a leading cause. Given the eminent threat infectious diseases pose to amphibian populations, there is a need to understand the host-pathogen-environment interactions that govern amphibian susceptibility to disease and mortality events. However, using animals in research raises an ethical dilemma, which is magnified by the alarming rates at which many amphibian populations are declining. Thus, *in vitro* study systems such as cell lines represent valuable tools for furthering our understanding of amphibian immune systems. In this review, we curate a list of the amphibian cell lines established to date (the amphibian invitrome), highlight how research using amphibian cell lines has advanced our understanding of the amphibian immune system, anti-ranaviral defence mechanisms, and *Batrachochytrium dendrobatidis* replication in host cells, and offer our perspective on how future use of amphibian cell lines can advance the field of amphibian immunology.

Keywords: Amphibians; Cell lines; Immune Response; Ranaviruses; Frog virus 3; Chytridiomycosis

1. Introduction

Many amphibian populations are declining worldwide, with the global assessment of amphibians by the International Union for Conservation of Nature (IUCN) identifying over one-third of species as threatened (Baillie et al., 2004). While the etiology is complex and can vary across species, six leading causes of current amphibian declines have been identified: land use change/habitat loss, commercial use, introduced species, contaminants, climate change, and emerging infectious diseases (reviewed by Collins, 2010). Chytridiomycosis, caused by the fungal pathogens *Batrachochytrium dendrobatidis* (Bd) and *Batrachochytrium salamandrivorans* (Bsal) (reviewed by Rollins-Smith and Le Sage, 2021; Fisher et al., 2021; Fisher and Garner, 2020), and ranaviruses, caused by ranaviruses such as frog virus 3 (FV3) (reviewed by Chinchar, 2002; Chinchar et al., 2017), are emerging infectious diseases of concern to amphibian populations. Recently, severe Perkinsea infections caused by protists have been associated with mass tadpole die-offs (Chambouvet et al., 2015; Isidoro-Ayza et al., 2017; and reviewed by Itoiz et al., 2022) and may represent another infectious disease of amphibian concern. Given the eminent threat infectious diseases pose to amphibian populations, there is a need to understand the integrated host-pathogen-environment interactions occurring at all levels of biological organization (whole animal, cellular, and molecular) that govern amphibian susceptibility to disease and mortality events.

To elucidate host-pathogen interactions, we must study processes in the complex context of a living host that represents the integration of all physiological functions in response to pathogens. However, this conflicts with increasing ethical considerations for the use of animals in research and global amphibian declines. The use of *in vitro* study systems to investigate host-pathogen-environment interactions has many advantages, including the ability to dissect mechanisms of host-pathogen interactions, tightly control and standardize experiments to enhance reproducibility, increase throughput, and reduce ethical concerns, which is critical when working with threatened or endangered species. *In vitro* systems can include tissue culture, primary cells dissociated from tissues or isolated from blood or peritoneal exudate, and cell lines. While tissue culture and primary cell culture-based systems have a potential advantage of yielding responses that may approximate those *in vivo*, these systems usually require the sacrifice of the amphibian host and are finite in their use. Thus, amphibian cell lines, or the amphibian “invitrome” – a term coined by Bols et al. (2017) to describe a collection of cell lines around a theme – represent an

attractive *in vitro* system to study host-pathogen interactions through the provision of an abundant and continuous source of material. Amphibian cell lines can also permit genetic manipulation that is either extremely challenging or not currently feasible with whole animals, providing an important knowledge basis for future *in vivo* confirmatory studies. We propose that leveraging the expanding amphibian invitrome is critical to advancing our mechanistic understanding of host-pathogen-environment interactions. This approach will help minimize animal use and ethical considerations associated with using amphibian hosts leading up to *in vivo* investigations that are necessary to validate *in vitro* findings at the whole animal and community levels. Further, we see potential for using yet to-be-developed amphibian cell lines for *in vivo* investigations that will allow amphibian biologists to delve deeper into mechanisms of host-pathogen interactions occurring *in vivo*.

The aims of this review are three-fold. Firstly, we aim to curate knowledge on the current amphibian invitrome for scientists who may wish to use these as *in vitro* study systems or create an amphibian cell line for use in their research. Secondly, we will highlight how the use of amphibian cell lines has advanced our understanding of host-pathogen interactions and uncovering mechanistic frameworks. Lastly, we offer our perspective on how future use of amphibian cell lines can advance the field of amphibian immunology.

2. Reported amphibian cell lines

2.1. Overview of amphibian cell lines

An in-depth literature search was conducted to curate a list of reported amphibian cell lines (**Supplemental Material 1**). The first amphibian cell line, FT, was developed from *Rana catesbeiana* tongue and reported in 1964 (Wolf and Quimby, 1964). This kickstarted an era of anuran cell line development in the late 1960s and early 1970s that generated several of the most popular cell lines including the *Xenopus laevis* kidney epithelial-like cell line A6 (Rafferty, 1969), which remains the most widely used amphibian cell line today. The amphibian invitrome continued to grow in the following decades, including the first caudatan cell line (TV1) reported in 1976 (Reese et al., 1976) (**Figure 1**). To date, there are at least 159 distinct amphibian cell lines (**Table 1, additional details in Supplemental Table 1**) originating from 23 species belonging to 5 families of anurans and 4 families of caudatans (**Figure 2A**). However, these numbers may be under representative as our search was limited to cell lines featured in English language

publications. Despite a healthy number of cell lines in the amphibian invitrome, there is a pronounced bias in favour of cell lines originating from *X. laevis* (**Table 1**) and less than 0.3% of the 8,498 known amphibian species are represented by existing cell lines, not to mention that over 100 new amphibian species are discovered each year (AmphibiaWeb, as of Oct 20, 2022).

Cell lines derived from anurans (141 in total) make up nearly 90% of the overall amphibian invitrome. Among these, 84 originate from *Xenopus* species, 47 originate from *Rana* species, and 10 originate from various other anuran genera (**Table 1**). As indicated in **Figure 2A**, this fails to capture the enormous diversity of the anuran order, representing only 5 of the 54 extant families. The presence of cell lines derived from members of Archaeobatrachia, Mesobatrachia, and Neobatrachia is a good start and may allow for some comparisons to be made between these suborders but does little to capture the enormous diversity within these clades. This is particularly true of Neobatrachia, which includes several families (Strabomantidae, 777 species; Microhylidae, 740 species; Rhacophoridae, 449 species) that lack cell lines despite containing a larger number of species than the Archaeobatrachia and Mesobatrachia suborders combined (27 and 355 species, respectively) (AmphibiaWeb, 2022). While there are only 18 cell lines derived from caudatans, cell lines have been developed from 4 of the 10 extant families (**Table 1**). The largest gap in the *in vitro* study of this clade is the absence of cell lines derived from the family Plethodontidae, which contains 495 species, constituting 64% of all extent caudatans. Lastly and most conspicuously, no cell lines originating from caecilians are known, meaning that one of the three extant orders of amphibians remains entirely unrepresented in the amphibian invitrome.

Collectively, cell lines in the amphibian invitrome originate from various developmental stages and diverse tissues (**Figure 2B**). Nearly a third of all amphibian cell lines have been established from whole embryo or whole larvae primary cultures of unknown tissue type. This is unsurprising as embryos were the cell source preferred by several pioneers of amphibian cell culture (Rafferty, 1965; Freed et al., 1969; Rafferty, 1969; Freed and Mezger-Freed, 1970; Freed, 1972) and embryos remain a reliable source of cell lines at the time of writing (e.g., Gorbisky et al., 2022). Cell lines derived from adult amphibians are often established from a defined tissue of origin, as specific organs or tissues can be more easily isolated for use in primary cell culture. Select tissues of origin are overrepresented in the amphibian invitrome due to methodological factors and the prolific work of researchers with a particular focus. This is exemplified among caudatan cell lines, where interest in the impressive capacity of these animals to regenerate limbs

has led to six cell lines derived from limbs or limb blastema, while only five cell lines are known to originate from other tissues (**Table 1**). Overall, the greatest number of cell lines have been developed from kidney tissue (24), with lines from several families of anurans and one caudatan (**Table 1**). Many kidney cells lines have been developed to study kidney tumours such as the Lucké Renal Carcinoma and their associated viruses (Rafferty, 1965; Wong and Tweedell, 1974; Tweedell and Williams, 1976). Ten cell lines have been developed from the retina and twelve from the thymus, although nearly all these cell lines have been produced from *X. laevis* through the work of Sakaguchi and colleagues (Sakaguchi et al., 1989; Sakaguchi and Henderson, 1993) or Du Pasquier, Robert and colleagues (Du Pasquier and Robert, 1992; Robert et al., 1994), respectively (**Table 1**). Liver, skin, muscle, spleen, bone, and larval tails have each been used to generate at least three cell lines through the efforts of multiple research groups (**Figure 2B**). Despite their different tissue origins many of these cell lines exhibit a nondescript epithelioid or fibroblastoid morphology, rather than resembling specialized, tissue-specific cell types. Most of the amphibian cell lines generated from primary cultures have not undergone a cloning step and thus likely represent cell lines of mixed cell types or subtypes versus clonal cell lines of uniform cell type. There are also varying standards of practice regarding diligent documentation of passage number which can introduce variability in cellular parameters due to changes in cellular characteristics or compositions of the cell line over time. While many tissues and cell types remain unrepresented, these diverse cell lines demonstrate a broad potential for cell line establishment across tissues and stages.

The amphibian invitrome includes all amphibian cell lines that have been developed. However, cell lines require specialized and often intensive maintenance and so it is helpful to further classify cell lines based on their storage and availability. Within the broader amphibian invitrome, cell lines can be classified into “curated”, “commercial”, “informally shared”, “zombie”, and “cryptic” invitromes (Bols et al. 2017). The “curated” invitrome includes cell lines held in the collections of formal agencies such as the American Type Culture Collection (ATCC) that are dedicated to cataloguing, distributing, and storing cell lines. Similarly, the “commercial” invitrome includes cell lines that can be purchased from commercial companies. The “informally shared” invitrome includes all cell lines that can be obtained via informal sharing between researchers. Lastly, the “cryptic” invitrome refers to cell lines which have been developed but have not appeared in publication, while the “zombie” invitrome refers to cell lines which have been

described in publications but do not appear to be used or stored any longer (Bols et al., 2017). The curated amphibian invitrome is minimal, with only 28 cell lines stored in repositories worldwide (**Supplemental Table 1**). Of the 28 curated cell lines, six are available from the ATCC, three from the European Collection of Authenticated Cell Cultures (ECACC), one from the German Collection of Microorganisms and Cell Cultures (DSMZ), one from the Italian Biobank of Veterinary Resources (IZSLER), one from the Kunming Cell Bank (KCB), ten from the *Xenopus laevis* Research Resource for Immunobiology (XLRRI) and 12 from the RIKEN Cell Bank (RCB). The RCB has the largest catalogue of amphibian cell lines and is the only repository that retains cell lines from caudatans. While some amphibian cell lines may be available for purchase from boutique commercial providers (e.g., kerafast), major life science suppliers do not presently stock such products. The informally shared and cryptic invitromes are by nature very challenging to quantify. Concerningly, the zombie amphibian invitrome encompasses most amphibian cell lines. Of the 159 amphibian cell lines identified, more than two thirds (109) are not known to be retained in any repository and have not been mentioned in publications within the last five years; thus, some or many of these cell lines may be lost (**Figure 2C**). While this will likely pose a major challenge for researchers hoping to obtain a particular amphibian cell line, the number of cell lines developed speaks to a wealth of cell line development methodologies that can be leveraged going forward.

Efforts to centralize knowledge on cell lines has led to 100 amphibian cell lines being listed in the cell line resource Cellosaurus (Bairoch, 2018; Cellosaurus, <https://www.cellosaurus.org/>, as of Oct 25, 2022). Cellosaurus was a key tool in our search and provides a searchable platform to centralize cell line information, including several cell lines which do not appear to have associated publications. As the catalogues of the various cell line repository organizations sometimes lack detailed cell line information and may have less than ideal search functionality, a robust, centralized catalogue of cell lines simplifies research considerably. Cellosaurus currently lists the widest array of amphibian cell lines for those looking to discover and compare cell lines. While many entries on amphibian cell lines provide few details, this could easily be remedied with increased input from cell line researchers. Cellosaurus has the potential to become a one-stop resource for identifying the existence and location of an amphibian cell line if it becomes more widely embraced by the community.

2.2. *Amphibian cell line establishment methods*

The methodologies used to generate cell lines from a diverse range of amphibian tissues have already been described comprehensively in several texts and method articles (Rafferty, 1969; Smith & Tata, 1991; Ferretti & Kumar, 2015; Strand et al., 2021), including a recent methodology paper from our group (Bui-Marinos et al., 2022). Our focus in this section is to outline trends in the reported amphibian cell line establishment methods that contextualize the current state of the amphibian invitrome, and to highlight methodological details that might be of concern for immunological studies.

2.2.1. *Primary culture and immortalization*

Primary cultures for cell line development are generally produced from amphibian tissue through one of two methods, tissue explant culture or chemical disassociation. Tissue explant culture involves mechanically cutting tissue into small segments which are placed in culture media to allow cells to migrate from the tissue segment into the culture vessel (e.g., Rafferty, 1969; Yuan et al., 2015; Bui-Marinos et al., 2020). Chemical dissociation of tissue employs chelators and/or enzymes to break up the extracellular matrix and produce a cellular suspension (e.g., Wolf and Quimby, 1964; Reese et al., 1976; Gorbisky et al., 2022). The most appropriate method needs to be empirically determined for each tissue. For example, experimentation with tissues from the newt *Notophthalmus viridescens* showed that heart tissue could be dissociated via mincing and trypsin digestion, and liver could be dissociated via mincing and collagenase digestion, but limb tissue was highly resistant to enzymatic digestion and cells could only be obtained by tissue explant culture (Ferretti and Brockes, 1988). Both methods have been used to generate cell lines with varied cell types, but the majority are adherent with an epithelioid or fibroblastoid morphology.

Once a primary culture has been produced, cells must be immortalized to generate a continuous cell line. Most of the amphibian cell lines described have immortalized spontaneously, arising naturally from subcultures of an initial primary culture (**Supplementary Table 1**). Several cases exist of cell lines arising in this manner from natural or induced tumours (Rafferty, 1969; Arthur and Balls, 1971; Tweedell and Williams, 1976; Fukui et al., 1992; Du Pasquier and Robert, 1992), but more often these lines originated from apparently healthy individuals and tissues. Only three amphibian cell lines have been immortalized through intentional intervention, and all have relied on transformation of the cell line to induce expression of the SV40 large T-antigen – these

include a *Bombina maxima* tadpole skin cell line and two *N. viridescens* limb blastema cell lines (Washabaugh, 1994; Xiang et al., 2012; **Supplementary Table 1**). While the success of SV40 large T-antigen-induced immortalization in both anurans and caudatans suggests the potential for widespread application, we were not able to find later references to these cell lines in the literature, therefore their long-term stability remains unclear. The potential for spontaneous immortalization of non-cancerous amphibian cells is also observed in fish (reviewed in Bols et al., 2017) and contrasts with mammalian cells that often require multiple transformations for immortalization (reviewed in Akagi, 2004).

2.2.2. Culture and characterization

Amphibian cell culture methods are simple and largely similar between cell lines. Nearly all culture media formulations consist of a common minimal medium such as Leibovitz's L-15, Minimum Essential Medium, or Medium 199 that has been diluted with sterile water to 0.6-0.8 × the standard concentration (to reflect amphibian osmolarity) and supplemented with 10-20% bovine serum. These media formulations have successfully supported primary cultures and established cell lines from both anurans and caudatans, as well as larvae and adults (e.g., Reese et al., 1976; Ferretti and Brockes, 1988; Vo et al., 2019c; Bui-Marinos et al., 2020). In some cases, cell-conditioned medium from another cell line or previous primary cultures has been used to support the growth of a primary culture or early cell line, although this is generally not necessary once an immortal cell line has been established (Anizet et al., 1981; Picard et al., 1983; Washabaugh, 1994; Gorbsky et al., 2022). The optimal incubation temperature for amphibian cell lines is typically around 25 °C, although cell lines may vary a great deal in their ability to grow at or tolerate a broad range of temperatures (e.g., Rafferty, 1969; Vo et al., 2019c; Bui-Marinos et al., 2020). Once a continuous cell culture has been established cloning can be used to generate cell lines originating from a single cell and may result in the cloning of multiple types or subtypes of cells originally in the continuous cell culture. This allows for the establishment of clonal cell lines with uniform cell types and clearly defined passage numbers from an initial mixed cell culture. Cloning has been applied only sparingly in the establishment of amphibian cell lines, most notably in the development of the various *Xenopus* lymphoid tumour cell lines (Du Pasquier and Robert, 1992; Robert et al., 1994). Many of the existing amphibian cell lines likely consist of a mixture of cell types and the passage number of the cell line from initial establishment from primary cultures

may or may not be known and may lead to variability in cellular responses depending on the passage number of the culture and how stable the cell line is.

The extent to which amphibian cell lines have been characterized varies, and in many cases only consists of a visual assessment of their morphology. The vast majority of described amphibian cell lines present an epithelioid (74) or fibroblastoid (38) morphology (**Table 1**). Cell lines resembling endothelial cells (Mawaribuchi et al., 2008), ameboid cells (Park et al., 2000), chondrocytes (Conceição et al., 2013), hepatocytes (Chang and Shapiro, 1990), lymphoid cells (Du Pasquier and Robert, 1992; Robert et al., 1994), thrombocytes (Sugimoto, 2015), myoblasts (Yaoita and Nakajima, 1997), mesenchymal stromal cells (Ferretti and Brockes, 1988), and melanophores (Okumoto, 2001) have also been described, but are generally unique or represented by a small group of related cell lines (**Table 1**). Karyotype analysis has historically been a popular method of cell line characterization and continues to be used to assess cell line stability (Freed et al., 1969; Rafferty, 1969; Gorbsky et al., 2022). While karyotyping can reveal chromosomal abnormalities that might contribute to cell line immortalization (Ellinger et al., 1983; Goyos and Robert, 2009; Sinzelle et al., 2012), many examples of stable euploid cell lines exist, thus it is not a consistently insightful method of characterization. Analysis of senescence associated β -galactosidase activity has become an additional practice to assess cell line immortalization in recent years (Vo et al., 2019c; Bui-Marinos et al., 2020; Gorbsky et al., 2022), while older studies relied on long term culture of the cell line, often propagating a line for hundreds of passages and observing for changes in growth rate and cell morphology. Several recently established cell lines have been confirmed to belong to their suspected species of origin using DNA barcoding of the mitochondrial cytochrome *c* oxidase subunit I gene (Vo et al., 2019a; Vo et al., 2019c; Bui-Marinos et al., 2020). In several cases, RT-PCR analysis targeting a panel of cell or tissue specific transcripts has been used to identify the cell type of a cell line (e.g. Conceição et al., 2013; Bui-Marinos et al., 2020). This method has been limited to cell lines derived from *X. laevis*, as other species have historically lacked the genetic resources to design comprehensive panels and have been limited to analysis of a small number of genes of particular interest. This may be changing however, as RNA-seq and transcriptome analysis continue to become more common, allowing for the design of gene-specific RT-PCR primers in non-model organisms and even whole-transcriptome characterization of cell lines (Gorbsky et al., 2022; Zhang et al., 2022). Several challenges remain, as the functions of molecular markers characterized in humans or mice may

not be conserved in amphibians, and *de novo* transcriptome assembly and annotation require a high degree of technical expertise. Despite these hurdles, transcriptomic methods have the potential to improve cell line characterization by facilitating more extensive characterization, including identification of immune transcripts (e.g., pathogen recognition receptors, major histocompatibility complex) that could serve as a starting point for additional functional characterization.

3. Use of cell lines to elucidate amphibian immune functions/mechanisms

Amphibian cell lines have been used for immunological study since they were first established. This has included examination of immune cell function and development, investigation of cellular responses to immune stimuli including pathogen-associated molecular patterns and host cytokines, and most recently, modelling interactions with emerging viral and fungal pathogens. We discuss the contribution of amphibian cell lines to our understanding of amphibian immunology and provide a summary of recent advances from *in vitro* models (Figure 3). Currently, very few cell lines of hematopoietic origin have been developed, preventing the study of many specialized immune functions, but the few immune cell lines which exist have been well studied, and non-immune cell have been used to study several more ubiquitous immune defense mechanisms.

3.1. Amphibian immune cell lines

Immune cells of the hematopoietic lineage are of interest due to their highly specialized immune functions and the central role they play in immune responses at an organismal level. Unfortunately, despite their potential utility, cell lines of hematopoietic origin have proved to be quite difficult to generate. At the time of writing, the lymphoid tumour cell lines developed by Louis Du Pasquier, Jacques Robert, and colleagues at the Basel Institute for Immunology are the only amphibian cell lines known to belong to this lineage. The long search for a lymphoid tumour in amphibians, and the process of developing the first lymphoid tumour cell line are described by Du Pasquier and Robert in their initial publication (Du Pasquier and Robert, 1992). All these cell lines originated from apparently spontaneous lymphoid tumours of adult *X. laevis* and exhibit an unusual phenotype resembling T- and B-cells, possessing molecular markers indicative of both cell types as well as undifferentiated lymphocytes (Du Pasquier and Robert, 1992; Robert et al., 1994). Despite all the lymphoid cell lines originating from adults, all but the ff-2 cell line lack cell

surface expression of MHC class I, typical of a larval phenotype (Robert et al., 1994). These unusual qualities have been explored in numerous impactful studies in the 20 years since the first lymphoid tumour cell lines were developed.

The *X. laevis* lymphoid tumour cell lines have been valuable tools for investigating amphibian cancer immunology and T-cell development. They have been used to investigate key differences in tumour tolerance between the larval and adult immune response in *X. laevis* (Robert et al., 1994; Robert et al., 1995), to identify novel apoptosis related proteins (Horton et al., 1998), to explore the expression and anti-tumour role of the heat shock protein gp96 (Robert et al., 1999; Goyos et al., 2004), and to demonstrate the role of non-classical MHC class Ib molecules in anti-tumour responses and tumour immune evasion (Goyos et al., 2007; Haynes-Gilmore et al., 2014). Most recently, the lymphoid tumour cell lines 15/0 and ff-2 continue to further our understanding of cancer immunology by modeling the development and infiltration of semi-solid tumours (Haynes-Gilmore et al., 2015), and investigating how innate-like T-cells contribute to tumour tolerance and rejection (Banach et al., 2019). Lymphoid tumour cell lines have also been used to clone and characterize the *Xenopus* β 2-microglobulin (Stewart et al., 2005), and to demonstrate the role of non-classical MHC class Ib molecules in T-cell development (Goyos et al., 2009). One major example of a discovery made using lymphoid tumour cell lines was the discovery of CTX (Chretien et al., 1996), a cell surface marker of cortical thymocytes in *Xenopus*. The B3B7, ff-2, ff-2.64, and 15/40 cell lines were integral to the discovery of CTX and later experiments, which showed that crosslinking CTX resulted in abnormal mitosis, multinucleation, and growth inhibition, demonstrating its role in regulating cell replication (Robert et al., 1997a; Robert et al., 1997b). CTX was later shown to be a member of a family of immunoglobulin-like proteins conserved across vertebrates (Chretien et al., 1998), and members of this family have since been linked to cancer in humans (Scanlan et al., 2006). The varied and fruitful applications of lymphoid tumour cell lines highlight the value of hematopoietic immune cell lines and tumour cell lines in general, and they have contributed to the use of *X. laevis* as a model organism for the study of cancer immunology.

3.2. Cellular responses to stimuli

In the interest of better understanding amphibian immunology, any cell line may be valuable depending on the question being asked. There has been recent recognition in the field of

immunology that all cells have immune function in that all cell types participate in the recognition of, and response to, pathogen- and damage-associated molecules to some degree. Recognition of non-self or damage-associated molecules by cellular receptors is typically followed by a cascade of cellular reactions leading to the production and release of cytokines, which kickstarts a broader immune response tailored to the insult detected. Identifying the cellular receptors that recognize pathogen-associated molecular patterns (PAMPs), and the intracellular signalling molecules that convey the stimulus, is essential to understand the initiation of an immune response. Similarly, identifying cytokine receptors and their associated intracellular messengers is necessary to understand how that response propagates within and between cells and through the broader organism. While the various receptors and intracellular messengers have been well studied in humans and mice, and many orthologs have been identified in amphibians, little mechanistic research has been performed in amphibian systems. It should be kept in mind that the expression of many cytokines and cytokine receptors is limited to particular immune cell types and/or cellular activation states *in vivo* and observed expression of these genes in a cell line may be an artifact of gene deregulation rather than a genuine function of that cell type. However, with appropriate consideration, selection of a suitable cell line(s) can provide a simplified model to investigate the effects of isolated pathogen molecules and host factors on cellular immune responses, allowing for deeper insight and comparison.

3.2.1. *Response to PAMP treatment*

Researchers commonly use commercially available immune stimulants that have either been purified from a pathogen or are synthetic analogues that approximate PAMPs (but are not necessarily identical) to study cellular immune responses. Some of the more common immunostimulants used in the field are polyinosinic:polycytidylic acid [poly (I:C)], lipopolysaccharide (LPS), and di- and tri-acylated lipopeptides (e.g., Pam2CSK4 and Pam3CSK4). While we have included a summary of the research on LPS-treated amphibian cell lines for completeness, it should be noted that these cellular responses are likely not attributable to LPS, or at least not to recognition through toll-like receptor 4 (TLR4; discussed below).

In mammalian models, double stranded RNA (dsRNA), a viral PAMP, is detected by retinoic acid-inducible gene-I-like receptors (RLRs) in the cytoplasm or bound by scavenger receptors on the cell membrane and trafficked to the endosome where dsRNA is detected by toll-like receptor 3 (TLR3) (Limmon et al., 2008; reviewed by Chen and Hur, 2022). Response to

dsRNA has been investigated in several frog cell lines through extracellular exposure to the synthetic viral dsRNA analogue, poly(I:C). The work of Sang et al. (2016) showed that stimulation of *X. laevis* A6 cells with poly(I:C) resulted in significantly increased transcript levels for several interferon (IFN) genes, including members of both the type I and type III families. Vo et al. (2019a) demonstrated that BufoTad, an endothelial-like cell line derived from an *Anaxyrus americanus* tadpole, could bind poly(I:C) via Class A scavenger receptors (SR-A), agreeing with observations from mammals (Limmon et al., 2008). Treatment of BufoTad cells with poly(I:C) induced expression of an Mx-like gene, suggesting that the cell line was able to recognize the poly(I:C) and mount an IFN response leading to upregulation of IFN-stimulated genes (ISGs). Treatment of BufoTad cells with 0.1 µg/mL poly(I:C) also resulted in a significant reduction in the rate of cellular metabolism, while concentrations above this level appeared to be cytotoxic. We have shown that Xela DS2 and Xela VS2, epithelial-like cell lines derived from the dorsal and ventral skin of *X. laevis*, respond to treatment with poly(I:C) with increased transcript levels of type I IFN genes, the ISG protein kinase R (*pkr*), and proinflammatory cytokine genes (*tnf*, *il1b*, *cxcl8a*) (Bui-Marinos et al., 2020), indicating a cellular antiviral response similar to that observed in the BufoTad cell line. Although gene regulatory responses of Xela DS2 and Xela VS2 were similar following poly(I:C) treatment, Xela DS2 appeared to have a robust and sustained increase in measured transcript levels while Xela VS2 cells generally displayed a time-dependent increase in transcript levels. Further experimentation showed that poly(I:C) was also cytotoxic to Xela DS2 and Xela VS2 at concentrations of 1 µg/mL or more (Bui-Marinos et al., 2021) and suggested that some anuran cells may be more sensitive to poly(I:C) than other animals. Most recently, we used the Xela DS2 cell line to uncover *X. laevis* microRNAs that are differentially expressed in response to poly(I:C) (Todd et al., 2021). Small RNA-seq of Xela DS2 cells treated with poly(I:C) revealed the differential expression of 65 microRNAs, many of which were predicted to target regulators of antiviral pathways such as cGAS-STING, RIG-I/MDA-5, TLR signaling, and type I IFN signaling, as well as products of these pathways [nuclear factor-κB (NF-κB)-induced and ISGs]. These findings suggest that frog microRNAs are involved in frog cellular antiviral responses to viral dsRNA. This building body of research suggests that the ability to detect and respond to synthetic analogues of viral dsRNAs is conserved in anuran cell lines, but questions remain regarding the underlying mechanisms, how these responses might vary between cell types, and whether these lessons can be applied to other orders of amphibians. As all the studies above relied

on extracellular stimulation with poly(I:C), little is known about the RLR-dependent response to dsRNA in amphibians. Studies employing direct transfection of poly(I:C) into amphibian cell lines may be helpful in investigating RLR-mediated antiviral pathways.

LPS, a PAMP derived from the outer membrane of Gram-negative bacteria, is bound by LPS binding protein (LBP), transferred to CD14 which then presents LPS to the TLR4 - myeloid differentiation protein-2 (MD-2) complex in mammals, initiating NF- κ B signalling and an inflammatory response (reviewed by Park and Lee, 2013). Comparatively, studies have long indicated that fish and frogs are highly resistant to stimulation with LPS (Berczi et al., 1966), and experiments on *X. laevis*, *Rana pipiens* (Bleicher et al., 1983), *R. catesbeiana* (Roeder et al., 1989), and *Lissotriton helveticus* (Cornuau et al., 2014) have suggested that this is a widely conserved trait of amphibians. Studies in fish have confirmed that TLR4 does not function as a detector of LPS (Sepulcre et al., 2009), suggesting that responses to LPS in fish, and by extension amphibians, is likely due to contaminants in the LPS preparations used. Although responses to very high concentrations of LPS were observed to occur in some fish cells via a TLR4-independent mechanism (Sepulcre et al., 2009). Despite this, LPS has also been used to treat frog cell lines in attempts to stimulate them. Carter et al. (2002) first demonstrated that LPS treatment of XTC-2, a fibroblast-like cell line derived from an *X. laevis* tadpole, was cytotoxic and mediated by the activation of extracellular signal-regulated kinase 2 (ERK-2). In their investigation of the IFN response in *X. laevis*, Sang et al. (2016) also exposed A6 cells to LPS, which resulted in only very minor effects on IFN transcript levels at 5 h of exposure, but a significant upregulation in the transcript levels of several type I and type III family members following 24 h of exposure. Regulation of pro-inflammatory genes in response to LPS treatment was not examined by Sang et al. (2016). Interestingly, while TLR4 is present in *X. laevis* (Ishii et al., 2007), orthologs of the associated CD14 and MD-2 proteins have yet to be identified. These proteins are vital for detection of LPS in humans (da Silva Correia et al., 2001; Nagai et al., 2002). Although LPS treatments have been performed on several amphibian cell lines in attempt to stimulate an immune response, the potential for contaminated LPS combined with observations of high tolerance to LPS and the absence of CD-14 and MD-2 suggest that *X. laevis*, and possibly all anurans, either lack an LPS receptor or recognize this PAMP via an alternative, TLR4-independent mechanism.

In mammals a wide array of PAMPs consisting of proteins, polysaccharides, and compounds with diacyl or triacylglycerol moieties are detected by TLR2, either as a homodimer

or a heterodimer including TLR 1 or 6 (reviewed in Oliveira-Nascimento et al., 2012). Few examples exist of attempts to stimulate amphibian cell lines with TLR2 ligands. In their investigation of *X. laevis* IFN gene expression, Sang et al. (2016) also exposed A6 cells to the bacterial cell wall component lipoteichoic acid (LTA) and the synthetic lipopeptides Pam2CSK4 and Pam3CSK4, which are agonists of the TLR 2/2, 2/6, and 2/1 dimers, respectively. Pam2CSK4 induced a significant upregulation in *ifn* transcript levels when A6 cells were exposed for 24 h, while the other compounds failed to induce any significant upregulation. These findings suggest that the ligands differ in their ability to stimulate IFN gene expression, but whether this is due to innate differences in the downstream effects of the various TLR2 duplexes, or perhaps differential localization of these ligands or duplexes to the endosome, remains to be investigated.

In conclusion, relatively few studies have examined the response of amphibian cell lines to treatment with PAMPs, natural or synthetic. While there is a growing body of work using poly(I:C) to study antiviral responses, little is known about most other PAMP treatments aside from the fact that they do or do not stimulate a response, setting the scene for any number of mechanistic studies. Given that all but one of the studies mentioned above rely on *X. laevis* cell lines, it remains to be seen whether these responses are conserved in amphibians.

3.2.2. Responses to cytokine treatments

Experimental treatment of amphibian cells with known amphibian cytokines has rarely been performed and has been limited to cells and cytokines derived from *X. laevis*. Several *X. laevis* recombinant cytokines have been produced, including transforming growth factor beta 5 (TGF- β 5) (Haynes and Cohen, 1993), tumour necrosis factor (TNF) (Mawaribuchi et al., 2008), colony stimulating factor 1 (CSF-1) (Grayfer and Robert, 2013), interleukin 34 (IL-34) (Grayfer and Robert, 2014), and type I and type III IFNs (Grayfer et al., 2014; 2015). While all were shown to have a proliferative or immunomodulatory effect on amphibian cells, most studies of cytokine activity have relied on treatments applied to whole animals, or *in vitro* treatment of tissues or primary cells. The first study treating established amphibian cell lines with a recombinant host cytokine was conducted by Mawaribuchi et al. (2008) and focused on the effects of TNF on A6 and the *X. laevis* larval vascular endothelial cell line XLgoo. A6 cells were found to recognize recombinant *X. laevis* TNF through the endogenous TNF receptor, leading to activation of NF- κ B through interactions with Fas-associated death domain (FADD) and receptor-interacting protein 1 (RIP1). This was determined by transfecting A6 cells with expression vectors encoding non-

functional mutant FADD and RIP1 genes, as well as a luciferase reporter plasmid responding to NF- κ B activation (Mawaribuchi et al., 2008). XLgoo cells responded to treatment with recombinant *X. laevis* TNF by forming actin stress fibers and adopting a more elongated morphology, and these cells experienced a survival signal which repressed apoptosis induced by prior treatment with the thyroid hormone T₃ (Mawaribuchi et al., 2008). The A6 cell line has also been used to investigate the antiviral response stimulated by recombinant *X. laevis* type I and type III IFNs (Grayfer et al., 2014; 2015). Treatment with recombinant *X. laevis* type I IFN (rX/IFN) or the type III IFN (rX/IFN λ) significantly reduced replication of FV3 when cells were pretreated with the cytokines at concentrations of 5 ng/mL or greater, but rX/IFN treatment resulted in a significantly greater reduction in plaque forming units (PFU)/mL and FV3 vDNA Poll II copy number compared to rX/IFN λ . Additionally both cytokines stimulated an increase in relative transcript levels of interferon-induced GTP-binding protein Mx1 (*mx1*) and *pkrr* after treatment with 100 ng/mL, but only rX/IFN increased relative transcript levels of *mx2* (Grayfer et al., 2015). This data closely agreed with the upregulation of immune gene expression and reduction in FV3 replication observed *in vivo* when tadpoles were injected with rX/IFN or rX/IFN λ . These cell lines provided a platform to study the response to recombinant cytokines in a defined cell type and, in the case of TNF, allowed for mechanistic experiments relying on transfection of the target cell, demonstrating the value of cell lines when characterizing the response to extracellular messengers.

3.3. Pathogen interactions

Amphibian cell lines have been used as *in vitro* study systems to isolate and identify pathogens (e.g., isolate a new adeno-like virus associated with the Lucké tumour using the *Rana pipiens* WMPa cell line, Wong and Tweedell, 1974), assess host cell permissiveness and pathogen replication, model host-pathogen interactions, and characterize the role of pathogen genes in host immune evasion (highlighted within this section). These studies have revealed important lessons, including the need for diverse cell lines as some pathogens demonstrate strict host-cell susceptibility (e.g., Wong and Tweedell, 1974). Some amphibian cell lines have also been used to demonstrate the requirement of a fish host gene for pathogen susceptibility (e.g., Ma et al., 2018), investigate the destruction of cell monolayers by *Tetrahymena* sp. (e.g., Pinheiro and Bols, 2015), or propagate pathogens of invertebrates (e.g., tick *Arboviruses* in FTC-14, Pudney and Varma, 1971; Rift Valley fever virus in A6, Gaudreault et al., 2015, Rissmann et al., 2021). In this section,

we focus on the cell lines used to investigate host-pathogen interactions in the context of ranaviral (FV3; *Andrias davidianus* ranavirus, ADRV) and chytrid fungi (*Bd*, *Bsal*) infections. We note that cell lines of primarily *X. laevis* origin (A6, Xela DS2, Xela VS2) have been used to study anuran responses to FV3 and *Bd*, while cell lines from *A. davidianus* (i.e., GSM) have been used to study salamander responses to ADRV, despite the diversity of cell lines that exist in the amphibian invitrome.

3.3.1. *Ranaviruses*

3.3.1.1. *Frog virus 3 (FV3)*

FV3, the type species of the genus *Ranavirus* (family *Iridoviridae*), is a double-stranded DNA virus that infects a wide range of amphibian species, as well as some fish and reptile species. In susceptible amphibians, FV3 infection leads to edema, hemorrhage, and necrosis, and can result in mortality rates of over 90% (Daszak et al., 1999; Miller et al., 2007). FV3 infections have been linked to mortality events in numerous amphibian species across the Americas, Europe, and Asia (Marsh et al., 2002; Daszak et al., 2003; Stuart et al., 2004; Lesbarrères et al., 2012). Notably, ranaviruses including FV3 were found to be responsible for 43% of known amphibian die offs in the USA from 2000 to 2005 (Muths et al., 2006). Despite the devastating effects that FV3 infections can have on amphibian populations, our understanding of the amphibian-FV3 host-pathogen interaction remains incomplete. However, studies using amphibian cell lines have helped to formulate our current understanding of FV3 pathogenesis and amphibian antiviral responses.

FV3 was first isolated in 1966 from a homogenate of *R. pipiens* Lucké tumour and was initially cultured in fat head minnow (FHM) and baby hamster kidney (BHK 21/13) cell lines, as well as primary chick embryo cells (Granoff et al., 1966). While it is difficult to say for certain, FV3, or a closely related ranavirus, was likely the first amphibian pathogen used to experimentally infect an amphibian cell line. The *R. sylvatica* embryonic kidney cell line, KERS, the *R. pipiens* whole embryo cell line, WERPi, and the *R. pipiens* Lucké Renal Carcinoma cell line, LC-1, were experimentally infected with the ranaviral isolates FV-1 and FV-2 in 1965 (Rafferty, 1965), which had also been isolated by Granoff et al. (1965) and in the case of FV-1, could not be distinguished from FV3 in early characterization experiments (Granoff et al., 1966). While all frog cell lines tested were permissive to the ranaviruses, the rate of replication and cytopathic effect formation was greatest in KERS (Rafferty, 1965), already suggesting significant differences in

permissiveness between cell lines. While FV3 replication continued to be occasionally studied in amphibian cell lines such as the *X. laevis* whole tadpole cell line XTC-2 (Elliot et al., 1979), most of the early molecular and virological studies of FV3 continued to be carried out in fish and mammalian cell lines. However, an increasing number of recent studies have used amphibian cell lines to explore several facets of FV3 infection including the role of amphibian IFNs in antiviral defense, the receptors that support viral cell entry, and transcriptional responses to FV3, which we outline below.

The *X. laevis* A6, Xela DS2 and Xela VS2 cell lines have been used to investigate the role of IFNs in cellular antiviral responses to FV3. Pre-treatment of A6 with rX/IFN or rX/IFN λ resulted in increased expression of key ISGs and reduced viral loads, but this effect was less pronounced for rX/IFN λ (Grayfer et al., 2014; 2015). Interestingly, FV3 infection significantly increased *ifnl* transcript levels in both rX/IFN λ -treated and untreated A6 cells and reduced *pkrr* transcript levels in rX/IFN λ -treated cells (Grayfer et al., 2015). These observations suggested that FV3 may direct host cells toward an IFN λ response, which is less effective at minimizing viral replication. A6 cells have also been used to investigate the roles of secreted molecules from CSF-1- or IL-34-differentiated *X. laevis* macrophages in cellular restriction to FV3. Following infection, FV3 copy numbers were observed to be significantly lower in A6 cells pre-treated with supernatants obtained from IL-34-differentiated macrophages than those from CSF-1-differentiated macrophages (Yaparla et al., 2018). This effect was reduced in A6 cells treated with baricitinib, a Jak1/Tyk2 inhibitor, suggesting that IFNs were the causative factor. Transfection of A6 cells with antisense morpholinos targeting *ifnar2.1/2.2* and *ifnlr1* transcripts and co-incubation with recombinant extracellular domains of IFNAR2.1 and IFNLR1 also significantly reduced the effect, indicating that both type I and type III IFNs were contributing (Yaparla et al., 2018). Similarly, while Xela DS2 and Xela VS2 do not increase transcript levels for IFN and ISGs following FV3 infection, pre-treatment with 100 ng/mL of poly (I:C) conferred protection and provided evidence that prior induction of antiviral pathways is effective in restricting FV3 replication (Bui-Marinós et al., 2021). Considering these findings, we hypothesized that macrophages or other cell types in the (skin) tissue microenvironment might be responsible for initial production of IFNs that lead to induction of effective viral restriction to FV3 in other cell types, such as skin epithelial cells (Bui-Marinós et al., 2021).

FV3 is immunoevasive (reviewed in Grayfer et al., 2012) and cell lines provide an *in vitro* system to examine the role of FV3-encoded genes in evading cellular antiviral responses. A study of FV3 knockout mutants showed that several mutants were able to replicate at the same rate as wild-type FV3 in FHM (fish) and BHK-21 (hamster) cells yet replicated poorly in A6 cells, highlighting the importance of using an appropriate host cell for studies of viral pathogenicity (De Jesús Andino et al., 2015). Further investigation using the A6 cell line revealed that type I IFN transcript levels were significantly increased in cells infected with the 18K immediate-early protein FV3 knock out relative to cells infected with wild-type FV3. Furthermore, A6 cells infected with any of knockout mutants (Δ 18K, Δ 64R, Δ vIF-2 α , or Δ 52L) lacked the transient spike in type III IFN transcript levels observed in A6 cells infected with wild-type FV3 at 24 h post-infection (De Jesús Andino et al., 2015). An additional experiment observing the effect of rX/IFN pre-treatment on replication of FV3 mutants in A6 cells showed significantly lower rates of replication for FV3 mutants with 64R and vIF-2 α knockouts, indicating that expression of these genes is necessary to evade the IFN-stimulated antiviral response (De Jesús Andino et al., 2015).

Several amphibian cell lines have been used to study the role of Class A scavenger receptors (SR-A) in FV3 infection. SR-As are known to facilitate cellular entry of other DNA viruses such as adenovirus 5 and herpes simplex virus (Haisma et al., 2009; MacLeod et al., 2013) and were believed to have potential to facilitate cellular entry by FV3. Tadpole cell lines derived from *A. americanus* - BufoTad, *R. catesbeiana* – BullTad-leg, *R. clamitans* – GreenTad-HF2, and *R. sylvatica* – WoodTad-rpe, were profiled for the presence of SR-A transcripts using RT-PCR. Each cell line was confirmed to transcribe one or more SR-A genes and SR-A ligand binding blocking assays showed pre-treatment of cell lines with competitive SR-A ligands, but not non-competitive ligands, decreased cell-associated FV3 as measured by a qPCR (Vo et al., 2019b). While this data suggests that SR-As play a role in FV3 cell entry in these cell lines, our study of the *X. laevis* Xela DS2 and Xela VS2 skin cell lines indicated that Xela DS2 and Xela VS2 are permissive to FV3 despite lacking appreciable transcript levels for any of the SR-As examined (Bui-Marinos et al., 2021). Considering these findings, we proposed that FV3 entered the cells through the previously known mechanism of fusion at the plasma membrane or through receptor-mediate endocytosis using an alternative receptor. Despite an apparent absence of SR-As, our experiments using the Xela DS2 and Xela VS2 cell lines demonstrated that pre-treatment with poly(I:C) conferred a protective effect to subsequent FV3 challenge (Bui-Marinos et al., 2021),

likely because of the previously observed increases in type I IFN and ISG transcripts following poly (I:C) treatment of these cells (Bui-Marinis et al., 2020). As earlier work in the BufoTad cell lines had shown that pre-treatment with competitive SR-A ligands blocked the increased ISG transcript levels stimulated by poly(I:C), this suggests that cell lines may differ in their reliance on SR-As to activate antiviral responses as well.

Most recently, amphibian cell lines have been used to investigate the role of host and viral non-coding RNAs in FV3 infection and immunoevasion. Our small RNA-seq experiments performed using the Xela DS2 cell line have identified 49 different host microRNAs that are differentially expressed by the cell line in response to FV3 infection, 17 of which had not been previously identified (Todd et al., 2021). The microRNA expression profile of FV3-infected cells differed considerably from cells which had been treated with the synthetic viral dsRNA analogue poly(I:C), having fewer differentially expressed microRNAs overall. Many of the microRNAs found to be differentially expressed during FV3 infection were predicted to target transcripts encoding members of several key antiviral pathways, including cGAS-STING, RIG-I/MDA5, TLR3, TLR4, TLR7, TLR8, TLR9, and type I IFN signaling pathways, as well as several NF- κ B-induced genes and ISGs. Interestingly, FV3 infection resulted in suppression of *dicer1* and *drosha* transcript levels, suggesting that FV3 infection suppresses microRNA biogenesis (Todd et al., 2021). In a concurrent study, whole transcriptome analysis of tissues from FV3-infected *X. laevis* revealed numerous putative microRNAs encoded by the FV3 genome (Tian et al., 2021). Many of these putative viral microRNAs were predicted to target the 3' untranslated regions of IFN receptor beta subunit transcripts or IFN regulatory factor transcripts. To determine whether the putative microRNAs were able to regulate their predicted targets, a small number of siRNAs with sequences identical to putative miRNAs were synthesized and transfected into A6 cells. Transfection with these siRNAs resulted in reduced levels of IFN receptor beta subunit and IFN regulatory factor transcripts, validating the regulatory effect of the putative microRNA sequences (Tian et al., 2021). These studies continue the long history of applying amphibian cell lines to study host-FV3 interactions and highlight an exciting new realm of investigation for those studying amphibian immune regulation.

3.3.1.2. *Andrias davidianus* ranavirus (ADRV)

ADRV, also known as Giant salamander iridovirus (GSIV), is a member of the genus *Ranavirus* and has been reported to cause disease outbreaks in wild and captive Chinese Giant

Salamander (*A. davidianus*) that can result in up to 100% mortality of infected juvenile and adult hosts (reviewed by Jiang et al., 2021). The *A. davidianus* antiviral response to ADRV has been previously reviewed (Jiang et al., 2021), highlighting the contribution of both innate and adaptive immune responses. This body of research has largely studied host-ADRV interactions using *in vivo* challenge and *in vitro* primary culture approaches. However, the development of multiple cell lines from the Chinese Giant Salamander, including epithelioid cell lines derived from the thymus (GSTC), spleen (GSSC), kidney (GSKC) (Yuan et al., 2015), and muscle (GSM) (gift from Qi-Ya Zhang as reported in Chen et al., 2015), have facilitated the study of ranavirus replication in *A. davidianus* cells and *A. davidianus* cellular responses to ranavirus infection.

A. davidianus cell lines have proved useful to study replication of ranaviruses in host cells. Development of the first *A. davidianus* cell lines, GSTC, GSSC, and GSKC, permitted susceptibility testing to ADRV and demonstrated that while all three cell lines were susceptible to ADRV, GSTC permitted the highest level of viral replication (Yuan et al., 2015) and has since been used to perform viral titre assays for ADRV (Ke et al., 2018). Interestingly, all these cell lines are epithelial-like and were established from the same individual (Yuan et al., 2015), suggesting that susceptibility to ADRV can vary based on tissue origin of the cell line and exemplifying the need for a multitude of cell lines from the same species for successful viral propagation and host-pathogen interaction studies. ADRV-infected GSTC have also been used for ultrastructural characterization of viral replication, showing nuclear deformation, low-density viromatrix regions with few viral particles and crystalline arrays of many viral particles outside of the viromatrix (Yuan et al., 2015; Ke et al., 2018). Studies using cell lines have further extended their scope to support investigation of host cell susceptibility to interspecies pathogens. For example, the *Rana grylio* virus (RGV), isolated from a diseased *Rana grylio* frog, was found to replicate faster and to a higher level than ADRV in GSTC cells and supported *in vivo* observations of higher RGV replication and fewer differentially expressed immune genes in response to interspecies infection (Ke et al., 2018). These cell lines could prove useful for future study of recombinant ADRVs to characterize the effect of viral gene deletion on viral replication and to elucidate the molecular basis for viral restriction in cells of different types and/or origins.

Research on the salamander immune system and response to pathogen challenge is still in its infancy. However, recent studies on *A. davidianus* challenged with ADRV have provided insight on antiviral responses (reviewed by Jiang et al., 2021). *In vivo* studies of ADRV-infected *A.*

davidianus have provided evidence of causal biological activities, such as tissue-specific regulation of gene transcription in response to ADRV infection. Transcripts for pattern recognition receptors involved in pathogen recognition such as toll-like receptor 7 (*tlr7*) and the RIG-I-like receptors RIG-I (*rigi*) and melanoma differentiation-associated protein 5 (*mda5*; formally named as IFN-induced, with helicase C domain 1, *ifih1*) were increased in ADRV-infected individuals. Furthermore, ADRV infection was associated with increased transcript levels of type I IFN and downstream ISGs such as *mx* genes (reviewed in Jiang et al., 2021). These *in vivo* causal studies were complemented with *in vitro* studies using primary cultures and cell lines. Overexpression of *A. davidianus* type I IFN (*ifn*) in GSM cells challenged with GSIV coincided with higher transcript levels of an ISG, *mx*, and with decreased viral transcription and viral titres (Chen et al., 2015), demonstrating the involvement of type I IFNs in the salamander antiviral response. Subsequent studies further investigated the role of ISGs, such as *mx* and oligoadenylate synthetase (*oas*) in the salamander antiviral response to ADRV. These two studies observed *mx* and *oas* transcript levels to increase over 72 h in GSM cells in response to ADRV infection, reminiscent of kinetics observed in tissues from *in vivo*-infected individuals, and overexpression of the *mx* or *oas* genes in GSM cells similarly reduced ADRV viral replication (Liu et al., 2020, Zhang et al., 2022). All three studies used fluorescent protein fusion constructs to confirm overexpression of *ifn*, *mx* or *oas* and to provide additional information on the subcellular localization of these recombinant antiviral molecules in GSM cells (Chen et al., 2015; Liu et al., 2020). The suitability of the current giant salamander cell lines, particularly GSM, to genetic manipulation has provided initial mechanistic data on the role of key genes in the protective antiviral response to ADRV and will be essential to continue advancing our understanding of the contribution of salamander host antiviral molecules to anti-ADRV defences and ADRV-encoded molecules to immunoevasion.

The GSM cell line has been instrumental to investigating the mechanism of cell death following ADRV infection (Li et al., 2019; 2021a; 2021b). An initial study demonstrated ADRV-infected GSM cells to exhibit hallmark characteristics of apoptotic cell death including DNA fragmentation, activation of caspases 3, 8 and 9, reduced mitochondrial membrane potential, cytochrome *c* release, and increased transcript levels of key genes involved in the apoptotic cell death pathway (Li et al., 2019). Using the GSM cell line, researchers further investigated the role of proapoptotic (Bid) and antiapoptotic (Bcl-xL) molecules in the B-cell lymphoma-2 (BCL-2) superfamily in the apoptotic mechanism of cell death in ADRV-infected cells (Li et al., 2021a;

2021b). Overexpression of Bid in ADRV-infected GSM cells resulted in enhanced ADRV replication, cytopathic effects and coincided with increased ADRV-induced apoptosis (Li et al., 2021a). Conversely, overexpression and siRNA-mediated knockdown of *A. davidianus* Bcl-xL in GSM demonstrated the role of Bcl-xL in limiting apoptosis in ADRV-infected cells through interaction with Bak, another BCL-2 superfamily member (Li et al., 2021b). These studies provide convincing evidence that ADRV-infected salamander host cells die through apoptotic cell death mechanisms.

Together, studies on amphibian cellular immune responses to ranaviral challenge demonstrate the utility of amphibian cell lines in understanding the role of individual genes in cellular responses to viral infection through characterization of functional cellular responses, overexpression and knockdown of target genes, identification and characterization of protein-protein interactions, and intracellular protein localization techniques. The ability to genetically manipulate amphibian cell lines will be particularly useful for future investigation of individual members of large gene families to ascertain their functional roles in cellular immune responses and host-virus interactions.

3.3.2. *Chytrid fungi*

As causal agents of the devastating chytridiomycosis disease, *Bd* and *Bsal* are considered major contributors to amphibian population declines (Lambert et al., 2020; Scheele et al., 2020). *Bd* was discovered in 1993 and has caused declines in hundreds of amphibian species across the globe (Berger et al., 1998). *Bsal* was discovered in 2013 and is thought to have originated in Asia but has been devastating fire salamander populations in Europe (Martel et al., 2013). While able to infect frogs and toads (anurans), *Bsal* appears to cause greater disease in tailed amphibians (caudatans) (Martel et al., 2013). Despite slight differences in their pathogenic mechanisms and pathologies, both *Bd* and *Bsal* infect keratinized amphibian skin tissue, generally leading to hypertrophy, hyperplasia, and hyperkeratosis of host skin cells. This results in lesions that compromise the epithelial barrier and disrupt osmoregulation, culminating in heart failure (Berger et al., 2005; Martel et al., 2013). The amphibian immune response to chytrid infection, summarized in several excellent reviews (Grogan et al., 2018; Voyles et al., 2011), is often ineffective and can lack robust inflammatory responses and adequate lymphocyte infiltration (Berger et al., 1998; Pessier et al., 1999). While our understanding of chytrid-amphibian interactions remains far from complete, studies using primary cell cultures and permanent cell lines have been crucial to

providing initial knowledge regarding chytrid infection dynamics (Van Rooij et al., 2010; Verbrugghe et al., 2019).

The first study to develop *in vitro* systems to model chytrid infection dynamics used primary keratinocytes and epidermal explants from *X. laevis*, *X. tropicalis*, and *A. cisternasii* (van Rooij et al., 2010). These systems demonstrated that *Bd* zoospores adhered to host cells within 1-h post-infection (hpi), and hyperkeratosis (thickening of the outer skin layer) was evident at 5 days post-infection (dpi). Building on the concept of modeling chytrid-amphibian interactions in keratinocytes, Verbrugghe et al. (2019) optimized an early infection model using primary *Litoria caerulea* keratinocytes. In primary keratinocyte cultures, it was demonstrated that contact between *Bd* spores and host cells occurs around 4 hpi, while internalization occurred after 24 hpi. While primary keratinocyte cultures and skin explants are generally only viable for a few days, the *Bd* lifecycle occurs over 4-5 days. Thus, a longer-term *in vitro* system was required to model the full *Bd* colonization cycle. The A6 *X. laevis* kidney epithelial cell line was used to recapitulate different stages of host cell infection and intracellular growth by *Bd* (Verbrugghe et al., 2019). The use of the A6 cell line in this study was also instrumental in generating a timeline of the process of *Bd* infection in epithelial cells. The authors discovered that germ tube formation was evident after 4 hpi, while germ tubes began penetrating host cells at 1 dpi. An intracellular swelling appeared at the end of germ tubes 1-2 dpi, giving rise to a thallus. Intracellular colonization began around 2-3 dpi, while maturation of intracellular thalli and the formation of large intracellular zoosporangia was evident at 3-4 dpi. At 4-5 dpi, formation of a discharge tube was apparent, through which fungal contents were released into host cells, triggering apoptotic cell death (i.e., caspase-3 activation). This study successfully modeled the *Bd* colonization cycle (adhesion, invasion, and maturation, and host cell death; Verbrugghe et al., 2019), which until this point had largely been visualized using light microscopy and transmission electron microscopy in skin explants (van Rooij et al., 2012). Deepening our understanding of later stage chytrid-amphibian infection dynamics on cellular and molecular levels will require the continued use of long-term cultures or permanent cell lines. The Xela DS2 and Xela VS2 skin epithelial-like cell lines that we have developed may represent an ideal system in which to perform future *Bd* colonization experiments, given the relevance of their tissue origin.

4. Benefits and limitations of cell lines

There are several advantages to the use of cell lines, from technical, cost, ethical, and research question standpoints. From a technical standpoint, cell lines are relatively easy to work with due to the variety of commercially available standardized cell culture reagents and the established protocols for culturing cell lines from a wide variety of species. In addition, few amphibians are commercially available, and many non-commercially available amphibians are difficult to breed and maintain in captivity due to long generation times, requirements for specific environmental conditions that are critical to their life histories but have yet to be effectively recapitulated in the laboratory (e.g., wood frogs freeze during winter months), or a simple failure to thrive under laboratory conditions. From a cost perspective, the generation and maintenance of cell lines is cost effective, and permanent cell lines can provide a near endless supply of research material. From an ethical standpoint, the use of cell lines lessens the use of animals. This is especially beneficial to the amphibian research community, as it permits the study of species that may be threatened or endangered in the wild without taking animals from sensitive wild populations. On a similar note, generating cell lines from amphibian species that are approaching extinction permits their preservation via biobanking and facilitates further research on the species long after its extinction (Strand et al., 2020). At present, ethics approval is not needed for growing amphibian cell lines, thus making it relatively easy for laboratories to adopt the use of cell lines in their research. Cell lines can also provide unique research benefits. They can represent a homogenous cell population, which strengthens reproducibility of results and permits the study of individual cell types. In addition, cell lines facilitate high throughput screening studies that would otherwise be impossible given the number of animals that would be needed instead. Amphibian cell lines can also be a rich source of purified proteins. For example, *X. laevis* tumour-derived lymphoid cell lines have high protein levels of several immunologically important molecules and thus have the potential to be a valuable source of these molecules (e.g., Du Pasquier and Robert, 1992). Amphibian cell lines can also be valuable sources of genetic material. For example, XTC cells have been used to amplify and clone genes (Haynes and Cohen, 1993). Lastly, the use of cell lines allows for the creation of stable mutant cell lines in which a particular gene of interest has been knocked out or a particular transgene is expressed and facilitates the dissection of molecular mechanisms. Genetic transformation is easier to perform in cell lines compared to whole animals. While newer methods such as CRISPR-Cas9 are improving our ability to genetically manipulate

amphibians such as *X. laevis* (i.e., Wang et al., 2015; DeLay et al., 2018), they only aid studies of amphibians that can be bred in the laboratory and are not of help to the other 8,400+ amphibians that exist and likely have diversity in immune systems/function. In addition to the creation of knock-out or knock-in cell lines, amphibian cell lines can be transfected with fluorescent markers and these cells injected into whole animals as a method to trace their fate *in vivo* (Tamura et al., 2015). For example, tagged myotubes were injected into regenerating Eastern newt (*N. viridescens*) limbs to elucidate their role in limb regeneration (Lo et al., 1993). While this is not an immunological application per se, it elegantly illustrates how cell lines could be used to study immune function or responses in whole animals *in vivo*.

While the use of cell lines presents numerous advantages, there are several drawbacks. First, while well suited to studies of the recognition and effector mechanisms that underly many immune functions, the use of cell lines does not capture the complex, integrated immune response that occurs at the organismal level that often involves cellular crosstalk within microenvironments, input from local and systemic soluble mediators, and the recruitment of additional cell types. Studying the responses of individual cell lines removes the context of the surrounding microenvironment and matrix that would be present in tissues, which may significantly alter cellular processes or fail to reproduce them (Risbridger, 2015). Similarly, the use of a cell line may be associated with an inherent inability to study cellular responses within a population of diverse cell types, which would be more representative of *in vivo* biology than studying one cell type in isolation. This may particularly be salient to the study of immune responses, given the importance of molecular interactions between different cell types. For example, studying lymphocytes in the absence of the antigen-presenting cells that activate them, such as macrophages, may lead to an incomplete picture of the immune process being studied. However, implementation of co-culture systems could help to mitigate this limitation. Secondly, researchers often assume a cell line comprises cells that are uniform, when in fact many cell lines are heterogenous and can even include stem/progenitor cells (Risbridger, 2015), although this may be an advantage depending on a researcher's goal (i.e. studying the interaction between these cell types/developmental stages). Cloning has seldom been applied to amphibian cell lines, meaning that many of the existing cell lines likely contain multiple cell types. As the number of passages from the initial founding cells is often unclear, shifts in the relative proportions or presence of these cell types can lead to variability in measured responses. Third, some cell lines have been known to change morphology,

behaviour, and even genotype as their passage number increases (Kaur and Dufour, 2012; Risbridger, 2015). Thus, it is important to monitor cell lines as they are passaged or set limits on the number of passages a cell line has undergone, as these changes can affect the behaviour of cell lines both within and between laboratories. Lastly, cell lines can be prone to viral and/or bacterial contamination that can persist in the cultures and can significantly affect research findings (Kaur and Dufour, 2012). This is a widespread problem, as it is estimated that 15-35% of cell lines are contaminated with mycoplasma (Kaur and Dufour, 2012). Aside from microbes, contaminants can include other cell lines. Contamination with a rapidly proliferating cell line can quickly take over the original cell line (Kaur and Dufour, 2012). Thus, while earlier papers may have utilized a “clean” cell line, later papers in the field that report using the same cell line may be referring to different cells entirely. A 2017 estimate suggested that over 32,000 papers have reported research with misidentified cell lines (Horbach and Halffman, 2017). A set of guidelines for the proper use of cell lines have been developed to increase awareness of contamination problems and to inform researchers how to deal with cell line contamination effectively (Geraghty et al., 2014). Thus, while the use of cell lines can be beneficial to advancing the fields of cellular and molecular biology, drawbacks must be considered when choosing *in vitro* systems.

5. How do we advance *in vitro* systems for studies of amphibian immune responses?

5.1. Perspective 1: Expand the repertoire of cell lines from other amphibian species

Generating cell lines from additional amphibian species, particularly from understudied clades, will better increase the species representation in the amphibian invitrome and has the potential to enhance comparative study between amphibian clades. As illustrated in **Figure 2a**, most amphibian families are not represented in the collection of existing cell lines, such as the caecilians for which no cell lines exist. Cell lines prepared from rare or understudied species serve as more manageable study systems of species that might be difficult to maintain in captivity. Cell lines would be particularly valuable for studies of species with populations threatened by pathogens, where an appropriate host cell line is needed to investigate host-pathogen interactions in a permissive, native environment but obtaining sufficient individuals to perform *in vivo* studies may be unfeasible. Furthermore, most studies of amphibian immune responses have used anurans, typically *X. laevis*, and have generally found a high degree of similarity to mammals and ray-finned fish. The limited studies which have been performed in caudatans suggest that there is a

considerable difference in immune functionality between these two amphibian orders, and likely between the caudatans and other vertebrate clades. Processes such as skin transplant rejection and lymphocyte replication in response to mitogens have been observed to be significantly slower in caudatans than the equivalent response in anurans (Cohen, 1970; Collins et al., 1975; Kinefuchi et al., 2013). Molecular analysis of the axolotl, *Ambystoma mexicanum*, has indicated that *A. mexicanum* has a normal level of T-cell receptor diversity (reviewed in Charlemagne et al., 1998) and a large number of MHC class I genes (Sammur et al., 1999) but that the species appears to have limited MHC class II β -chain diversity that may limit antigen presentation (reviewed in Tournefier et al., 1998). It remains unclear whether this is a condition specific to *A. mexicanum* or whether it may underlie the sluggish immune response typical of some caudatans. Until further comparative studies can be performed, any inferences about the caudatan immune response based on studies of anurans should be approached with appropriate skepticism.

While increasing representation of understudied groups in the invitrome is vital, there are also many gaps to fill among the more well studied species. Building on the tumour transplantation studies performed with *X. laevis* lymphoid tumour cell lines (Robert et al., 1994; Robert et al., 1995) and research tracking the fate of cultured cells in *N. viridescens* limb regeneration (Lo et al., 1993), the development of additional cell lines from commonly used laboratory strains such as the *X. laevis* J-strain could enable a plethora of adoptive transfer studies. The well characterized genome of the J-strain would also make experiments with these cell lines particularly convenient (Session et al., 2016). Similarly, *A. mexicanum* has a published genome (Smith et al., 2019) and, due to its established high regenerative capacity and tolerance of related tissue transplants (de Both, 1968; de Both, 1970), appears particularly well suited to adoptive transfer of cell lines. Performing adoptive transfer studies with transformed cell line strains (e.g., labeling with fluorescent markers, knockout of select cell surface receptors, reduced MHC type I expression, etc.) could provide insight into cell trafficking, receptor involvement in cell responsiveness, antigen presentation and more. Species suited to laboratory life such as *X. laevis* and *A. mexicanum* can be used to bridge the gap between *in vitro* and *in vivo* studies and can guide interpretation of findings from cell lines developed from non-model species for which *in vivo* experiments may not be feasible.

5.2. Perspective 2: Develop amphibian immune cell lines

We should strive to generate additional cell lines from leukocytes or their progenitors. While the existing *X. laevis* lymphoid tumour cell lines are a valuable resource (Du Pasquier and Robert, 1992; Robert et al., 1994), they cannot capture the incredible diversity of phenotypes and physiological responses present in the broader leukocytic lineage, herein referred to simply as immune cells. This leaves a gap in our understanding of both innate and adaptive immune cell function at a cellular level. While any additional cell lines of hematopoietic origin would be invaluable, we suggest the establishment of a monocyte/macrophage cell line from J-strain (or similar) *X. laevis* as a priority to facilitate *in vitro* and *in vivo* experimentation. Ideally, a monocyte cell line could be maintained and stimulated to differentiate into macrophages, which could then be used to study mechanisms of mononuclear differentiation, antimicrobial mechanisms (e.g., phagocytosis) or initiation of adaptive (e.g., antigen presentation to T-cells) responses. Methods for monocyte/macrophage cell line development may be adapted from existing work in teleosts. The development of the rainbow trout (*Oncorhynchus mykiss*) monocyte/macrophage-like cell line, RTS-11 (Ganassin and Bols, 1998) from spleen relied on methods comparable to those used for amphibians, including spontaneous immortalization. Several other monocyte/macrophage-like cell lines have been developed from other fish species using similar methods and incorporated Ficoll or similar density-based techniques to enrich mononuclear cells from disassociated head kidney (Dannevig et al., 1995; Rodriguez Saint - Jean et al., 2014; Wang et al., 1995; Wergeland and Jakobsen, 2001; Xia et al., 2021), or in a single case, peripheral blood (Faisal and Ahne, 1990). We have made attempts to spontaneously generate a mononuclear phagocyte cell line from *X. laevis* bone marrow samples. Some primary cultures initially resulted in a matrix of adherent stromal-like cells that supported suspension cell populations with cobblestone morphology. However, these cultures did not persist for any considerable length of time. Instead, many of these primary cultures ended up yielding fibroblast-like cell lines that appear to generate an extracellular rich cell-conditioned media, which may be useful for future attempts to initiate a mononuclear phagocyte cell line (unpublished work). The relatively low success rate of generating cell lines of hematopoietic origin appears to indicate that immortalization is a primary barrier to immune cell line development, so it may be necessary to employ targeted immortalization techniques, as suggested below.

Current amphibian cell line establishment methods tend to favour the generation of epithelioid and fibroblastoid cell lines (**Table 1**), and the long search for amphibian lymphoid tumours described by the authors that generated the lymphoid tumor cells lines (Du Pasquier and Robert, 1992; Robert et al., 1994) suggests that alternative methods to establish amphibian immune cell lines are needed. We propose that immortalization techniques should be attempted, such as transformation with the SV40 large T antigen that has been used extensively to immortalize cell lines (reviewed in Ahuja et al., 2005), including anuran and caudatan cell lines (Washabaugh, 1994; Xiang et al., 2012) outlined in this review. This transformation method appears to have potential for immune cell immortalization as it has been used to generate macrophage cell lines from humans and mice (Takayama, 1980; Nagata et al., 1983). However, additional transformation methodologies should also be trialed to determine the optimal methodology for a particular species/cell type. Transformation is not without drawbacks, as transformation inherently creates differences between the cell line and the primary cells that it was derived from, further straining any direct comparisons between the cell line and its native organism. While there is certainly room to discuss the pros and cons of a transformed cell line and how these might compare to a tumour cell line or a spontaneously immortalized cell line in practice, it stands that transformed cell lines can be very useful when properly applied, particularly if they are well characterized.

5.3. *Perspective 3: Characterize cell lines for the expression of immune-relevant genes*

Detailed characterization of existing and new cell lines is needed to determine the (immune) cell type(s) it resembles and which immunological mechanisms it can be used to model. For example, virtually none of the cell lines derived from whole tadpoles or embryos have been characterized for expression of immune genes. Transcriptome analysis is one method that could be more broadly embraced for cell line characterization. This method has been applied to several recently established cell lines, and it seems likely that this will become the standard should the cost of sequencing and computing resources continue to decline (Gorbsky et al., 2022; Zhang et al., 2022). While transcript levels do not necessarily directly reflect a cellular phenotype, the presence or absence of transcripts for known cell-type markers is useful in characterizing cell type or role in an immune response. As with model amphibians, few (if any) antibodies exist for non-model amphibians, and RT-PCR analysis is limited to targets that have been sequenced in a small number of distant relatives with well-conserved sequences, meaning that RNA-seq may be the

only viable option. In cases where preliminary characterization of the cell line suggests an immune functionality, it may be helpful to expose the cell line to a panel of commercially available PAMPs and/or immunostimulants and observe for a change in transcription levels, cell morphology, or cellular function associated with that (immune) cell type.

5.4. *Perspective 4: Three-dimensional in vitro culture systems*

We should build upon two-dimensional *in vitro* systems through co-culture and/or three-dimensional culture techniques to create enhanced *in vitro* systems to better reflect *in vivo* microenvironments. A sizable body of work addressing the development of cultures that more closely approximate tissues and organs exists in the mammalian cell culture literature, and a wide variety of methods have been developed, with organoids derived from differentiated stem cells currently considered to be the gold standard (reviewed in Fatehullah et al., 2016). While there is little precedent for three-dimensional cultures of amphibian cell lines, reviews of the mammalian methodology provide a wealth of techniques that could be adapted (Koledova, 2017; Hospodiuk et al., 2017). Examples of enhanced *in vitro* cell line-based systems could include layered fibroblast and epithelial cells to better approximate skin, combining hematopoietic and mesenchymal cells to model the hematopoietic niche, or introducing primary blood leukocytes to a structured culture of endothelial cells to simulate tissue infiltration.

6. **Concluding remarks**

Although the amphibian invitrome consists of over 150 cell lines, few have been used to investigate amphibian cellular immune responses. Nevertheless, the use of cell lines originating from *X. laevis* and *A. davidianus* have proved increasingly useful in advancing our understanding of amphibian host-pathogen interactions in the context of ranaviral and chytrid infections. Existing cell lines which have been historically used to study amphibian cellular immune function and host-pathogen interactions, and those that have yet to be used for such investigations, represent unlocked potential. In this review, we have provided our perspectives on four immediate areas of future research to unlock this potential, including the continued development and study of cell lines from understudied amphibians, increased focus on immune cell line development, greater cell line characterization, and application of three-dimensional culture methods. These proposed improvements to the amphibian invitrome and its use have the potential to transform our

understanding of amphibian immune responses at a mechanistic level while limiting use of live amphibians, particularly those from declining populations.

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8. Conflicting interests

The authors declare no conflicts of interest.

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1575

1577 Table 1. Reported amphibian cell lines.

Cell line	Parent	Species	Stage	Tissue of origin	Morphology	Reference
ACT1 (aka AL1)		<i>Ambystoma mexicanum</i>	Larva	Limb connective tissue	Fibroblast	(Roy et al., 2000)
BA 68.1		<i>Anaxyrus americanus</i>	Embryo	Unspecified	Epithelioid	(Freed et al., 1969)
BufoTad		<i>Anaxyrus americanus</i>	Tadpole	Whole	Epithelial/Endothelial	(Vo et al., 2019a)
GSKC		<i>Andrias davidianus</i>	Adult	Kidney	Epithelial-like	(Yuan et al., 2015)
GSM		<i>Andrias davidianus</i>	Unspecified	Muscle	Spindle-Shaped	(Chen et al., 2015)
GSSC		<i>Andrias davidianus</i>	Adult	Spleen	Epithelial-like	(Yuan et al., 2015)
GSTC		<i>Andrias davidianus</i>	Adult	Thymus	Epithelial-like	(Yuan et al., 2015)
Unnamed		<i>Bombina maxima</i>	Tadpole	Whole Skin (without tail)	Fibroblast	(Xiang et al., 2012)
Bok-2		<i>Bombina orientalis</i>	Embryo	Tail bud	Ameboid/Phagocytic	(Park et al., 2000)
Bor II		<i>Bombina orientalis</i>	Embryo	Whole	Epithelioid/Fibroblastoid	(Ellinger et al., 1983)
TB-6c		<i>Bufo marinus</i>	Unspecified	Urinary Bladder	Epithelial	(Handler et al., 1979)
TBM-54		<i>Bufo marinus</i>	Unspecified	Urinary Bladder	Epithelial	(Handler et al., 1979)
CPN4B		<i>Cynops pyrrhogaster</i>	Embryo	Whole	Epithelial-like	RCB Cat#: RCB1690
FUHEN		<i>Dryophytes japonicus</i>	Adult	Bone marrow	Thrombocytic	(Sugimoto, 2015)
HNS1A		<i>Hynobius nebulosus</i>	Embryo	Whole	Epithelioid	RCB Cat#: RCB0591
HNS2		<i>Hynobius nebulosus</i>	Embryo	Whole	Epithelioid	RCB Cat#: RCB0592
HNS3		<i>Hynobius nebulosus</i>	Embryo	Whole	Fibroblastoid	RCB Cat#: RCB0593
HNS4		<i>Hynobius nebulosus</i>	Embryo	Whole	Epithelioid	RCB Cat#: RCB0594
HNS5		<i>Hynobius nebulosus</i>	Embryo	Whole	Fibroblastoid	RCB Cat#: RCB0595
HTUD1		<i>Hynobius tokyoensis</i>	Embryo	Whole	Epithelioid	RCB Cat#: RCB0713
A1		<i>Notophthalmus viridescens</i>	Adult	Ankle	Fibroblastoid	(Ferretti and Brockes, 1988)
TV1		<i>Notophthalmus viridescens</i>	Adult	Dorsal iris	Epithelial	(Reese et al., 1976)
BIH1 / B1H1		<i>Notophthalmus viridescens</i>	Adult	Hind Limb Blastema	Fibroblastoid	(Ferretti and Brockes, 1988)
NvSV40-1		<i>Notophthalmus viridescens</i>	Unspecified	Limb Blastema	Fibroblastoid	(Washabaugh, 1994)
NvSV40-B3		<i>Notophthalmus viridescens</i>	Unspecified	Limb Blastema	Fibroblastoid	(Washabaugh, 1994)
TH4B		<i>Notophthalmus viridescens</i>	Adult	Thigh muscle	Mesenchymal	(Ferretti and Brockes, 1988)
A-11		<i>Pseudacris crucifer</i>	Hatching embryo	Whole	Mixed	(Rafferty, 1969)
A-4		<i>Pseudacris triseriata</i>	Hatching embryo	Whole	Mixed	(Rafferty, 1969)
LAH3		<i>Rana brevipoda</i>	Froglet	Dorsal skin	Epithelial-like melanophores	(Okumoto, 2001)
HO(6)		<i>Rana catesbeiana</i>	Unspecified	Unspecified	Unspecified	Cellosaurus: CVCL_Y444
FT		<i>Rana catesbeiana</i>	Adult	Tongue	Fibroblast	(Wolf and Quimby, 1964)
BullTad-leg		<i>Rana catesbeiana</i>	Tadpole	Unspecified	Fibroblastoid	(Vo et al., 2019b)
A-19		<i>Rana clamitans</i>	Hatching embryo	Whole	Fibroblast	(Rafferty, 1969)
A-20		<i>Rana clamitans</i>	Hatching embryo	Whole	Fibroblast	(Rafferty, 1969)
A-5		<i>Rana clamitans</i>	Hatching embryo	Whole	Fibroblast	(Rafferty, 1969)
GreenTad-gill		<i>Rana clamitans</i>	Tadpole	Gills	Epithelial-like	(Vo et al., 2019c)
GreenTad-HE4		<i>Rana clamitans</i>	Tadpole	Cephalic region	Epithelial-like	(Vo et al., 2019c)
GreenTad-HF1		<i>Rana clamitans</i>	Tadpole	Cephalic region	Fibroblast-like	(Vo et al., 2019c)
GreenTad-HF2		<i>Rana clamitans</i>	Tadpole	Cephalic region	Fibroblast-like	(Vo et al., 2019b)
GreenTad-HF3		<i>Rana clamitans</i>	Tadpole	Cephalic region	Fibroblast-like	(Vo et al., 2019c)
LAH1		<i>Rana nigromaculata</i>	Froglet	Dorsal skin	Mixed melanophores	(Okumoto, 2001)
LAH2		<i>Rana nigromaculata</i> (albino)	Froglet	Dorsal skin	Mixed melanophores	(Okumoto, 2001)
WERPa		<i>Rana palustris</i>	Embryo	Whole	Epithelioid	(Rafferty, 1965)
3AKRP		<i>Rana pipiens</i>	Adult	Kidney	Unspecified	(Gravell, 1971)
A-2		<i>Rana pipiens</i>	Hatching embryo	Whole	Epithelioid	(Rafferty, 1969)
A-3		<i>Rana pipiens</i>	Adult	Lucké tumor	Epithelioid	(Rafferty, 1969)
DRP 153	ICR-2A	<i>Rana pipiens</i>	Embryo, Androgenetic Haploid	Whole	Fibroblast	(Rosenstein, 1989)
DRP 36	ICR-2A	<i>Rana pipiens</i>	Embryo, Androgenetic Haploid	Whole	Fibroblast	(Rosenstein and Chao, 1985)
ICR B20	ICR-2A	<i>Rana pipiens</i>	Embryo, Androgenetic Haploid	Whole	Fibroblast	(Freed, 1972)

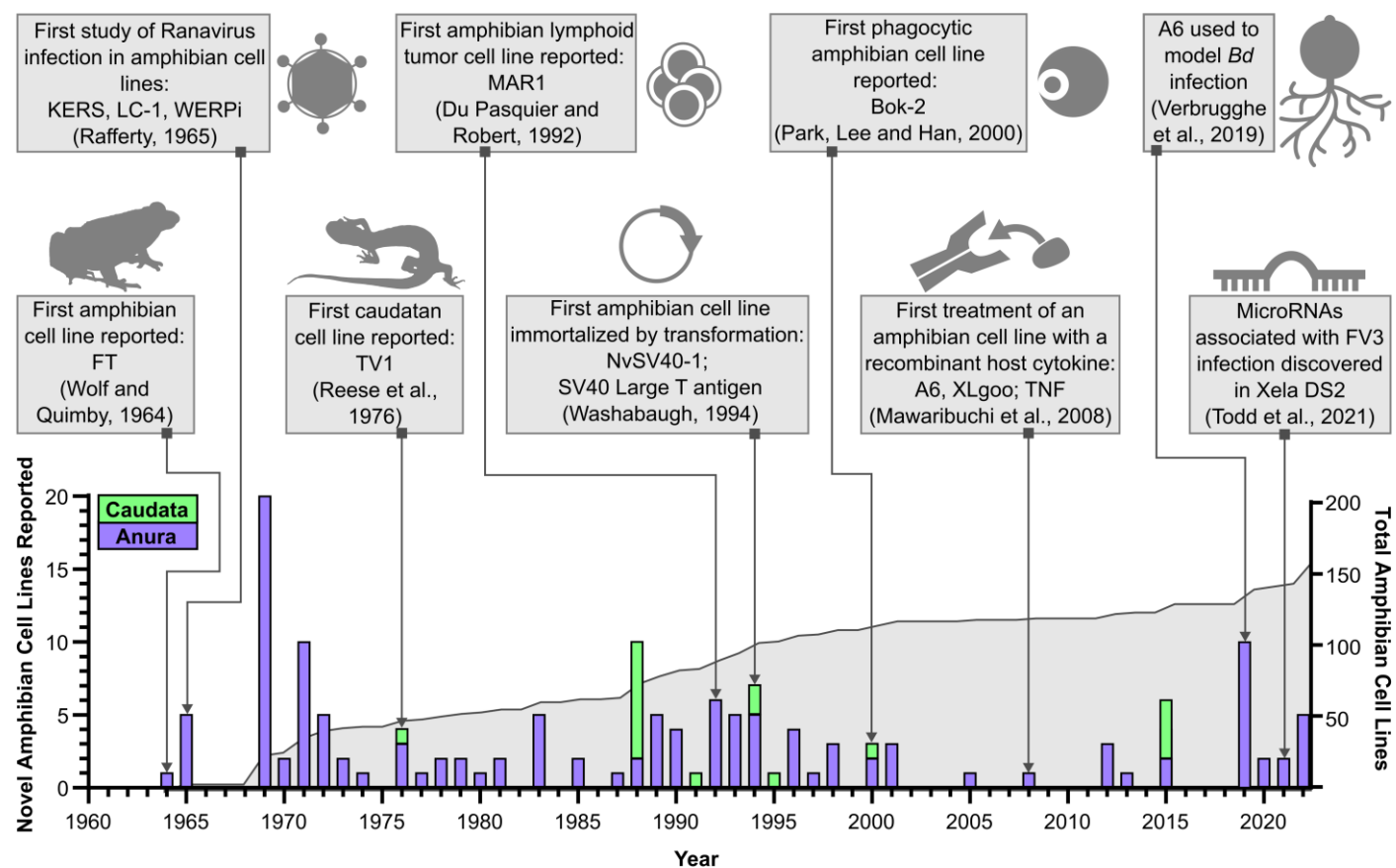
ICR B9	ICR-2A	<i>Rana pipiens</i>	Embryo, Androgenetic Haploid	Whole	Fibroblast	(Freed, 1972)
ICR-134 / RPH 67.134		<i>Rana pipiens</i>	Embryo	Whole	Fibroblastoid	(Freed et al., 1969)
ICR-2A1	ICR-2A	<i>Rana pipiens</i>	Embryo, Androgenetic Haploid	Whole	Fibroblast	(Freed, 1972)
ICR-2A2	ICR-2A	<i>Rana pipiens</i>	Embryo, Androgenetic Haploid	Whole	Fibroblast	(Freed, 1972)
PNKT-3		<i>Rana pipiens</i>	Embryo	Pronephric tumour	Mixed epithelioid/fibroblast	(Tweedell and Williams, 1976)
PNKT-4		<i>Rana pipiens</i>	Embryo	Pronephric tumour	Mixed epithelioid/fibroblast	(Tweedell and Williams, 1976)
PRKT		<i>Rana pipiens</i>	Embryo	Pronephric tumour	Mixed epithelioid/fibroblast	(Tweedell and Williams, 1976)
RPD 67.7		<i>Rana pipiens</i>	Embryo	Unspecified	Mixed epithelioid/fibroblast	(Freed et al., 1969)
RPH 67.194		<i>Rana pipiens</i>	Unspecified	Somite tissue	Mixed	(Freed et al., 1969)
WERPi		<i>Rana pipiens</i>	Embryo	Whole	Epithelioid	(Rafferty, 1965)
LC-1		<i>Rana pipiens</i>	Adult	Lucké Renal Carcinoma	Epithelioid	(Rafferty, 1965)
ICR-2A / RPH 68.2A		<i>Rana pipiens</i>	Embryo, Androgenetic Haploid	Whole	Fibroblast	((Freed and Mezger-Freed, 1970)
PNKT-4B		<i>Rana pipiens</i>	Embryo	Pronephric tumour	Unspecified	(Tweedell, 1978)
RPH 67.205		<i>Rana pipiens</i>	Embryo, Androgenetic Haploid	Whole	Epithelial-like	(Freed and Mezger-Freed, 1970)
RPKID		<i>Rana pipiens</i>	Unspecified	Kidney	Unspecified	(Tweedell and Lazar, 1992)
WMPa		<i>Rana pipiens</i>	Embryo	Kidney	Mixed epithelioid	(Wong and Tweedell, 1974)
A-1		<i>Rana sylvatica</i>	Large embryo	Kidney	Epithelioid	(Rafferty, 1969)
A-21		<i>Rana sylvatica</i>	Hatching embryo	Whole	Epithelioid	(Rafferty, 1969)
A-22		<i>Rana sylvatica</i>	Hatching embryo	Whole	Epithelioid	(Rafferty, 1969)
A-23F		<i>Rana sylvatica</i>	Hatching embryo	Whole	Epithelioid	(Rafferty, 1969)
A-23R		<i>Rana sylvatica</i>	Hatching embryo	Whole	Epithelioid	(Rafferty, 1969)
KERS		<i>Rana sylvatica</i>	Embryo	Kidney	Epithelioid	(Rafferty, 1969)
WERS		<i>Rana sylvatica</i>	Embryo	Whole	Epithelioid	(Rafferty, 1965)
WoodTad-Bone		<i>Rana sylvatica</i>	Tadpole	Hind leg bone	Fibroblast-like	(Vo et al., 2019c)
WoodTad-HE1		<i>Rana sylvatica</i>	Tadpole	Cephalic region	Epithelial-like	(Vo et al., 2019c)
WoodTad-rpe		<i>Rana sylvatica</i>	Tadpole	Retinal tissue	Epithelial-like	(Vo et al., 2019b)
XB691		<i>Xenopus borealis</i>	Tadpole	Whole	Epithelioid	(Picard et al., 1983)
XB693		<i>Xenopus borealis</i>	Tadpole	Whole	Epithelioid	(Picard et al., 1983)
XB693T		<i>Xenopus borealis</i>	Tadpole	Whole	Epithelioid	(Picard et al., 1983)
XB694		<i>Xenopus borealis</i>	Tadpole	Whole	Epithelioid	(Picard et al., 1983)
S3 / S3-1		<i>Xenopus laevis</i>	Embryo	Unspecified	Unspecified	(Cohen et al., 2000)
15/0		<i>Xenopus laevis</i>	Adult	Thymic Tumour	Lymphocytoid	(Robert et al., 1994)
15/0 (GFP+)	15/0	<i>Xenopus laevis</i>	Adult	Thymic Tumour	Lymphocytoid	XLRR1
15/0 (a haplotype class Ia+)	15/0	<i>Xenopus laevis</i>	Adult	Thymic Tumour	Lymphocytoid	XLRR1
15/0 (b2m knockdown)	15/0	<i>Xenopus laevis</i>	Adult	Thymic Tumour	Lymphocytoid	XLRR1
15/40		<i>Xenopus laevis</i>	Adult	Thymic Tumour	Lymphocytoid	(Robert et al., 1994)
A-24		<i>Xenopus laevis</i>	Adult	Ovarian tumour	Epithelioid	(Rafferty, 1969)
A6		<i>Xenopus laevis</i>	Adult	Kidney	Epithelioid	(Rafferty, 1969)
A6/C1	A6-2F3	<i>Xenopus laevis</i>	Adult	Kidney	Epithelioid	(Verrey et al., 1987)
A6-2F3	A6	<i>Xenopus laevis</i>	Adult	Kidney	Epithelioid	(Wills et al., 1992)
A6-KR	A6	<i>Xenopus laevis</i>	Adult	Kidney	Epithelioid	(Ellison et al., 1985)
A-7		<i>Xenopus laevis</i>	Adult	Kidney	Epithelioid	(Rafferty, 1969)
A8 2k		<i>Xenopus laevis</i>	Adult	Liver	Epithelioid	(Chinchar and Sinclair, 1978)
A8		<i>Xenopus laevis</i>	Adult	Liver	Epithelioid	(Rafferty, 1969)
A8W243	A8	<i>Xenopus laevis</i>	Adult	Liver	Epithelioid	(Griggs and Bender, 1972)
ACTK1		<i>Xenopus laevis</i>	Unspecified	Kidney	Unknown	KCB Cat#: KCB 82004YJ
B3.2		<i>Xenopus laevis</i>	Unspecified	Kidney	Unknown	(Seiler-Tuyns et al., 1988)
B3.2(tTA)1	B3.2	<i>Xenopus laevis</i>	Unspecified	Kidney	Unknown	(Camacho-Vanegas et al., 1998)
B3B7	MAR1	<i>Xenopus laevis</i>	Adult	Thymic Tumour	Lymphocytoid	(Du Pasquier and Robert, 1992)

B3D3	MAR1	<i>Xenopus laevis</i>	Adult	Thymic Tumour	Lymphocytoid	(Du Pasquier and Robert, 1992)
DWJ		<i>Xenopus laevis</i>	Unspecified	Unspecified	Fibroblast	XLRR1
DW15		<i>Xenopus laevis</i>	Unspecified	Unspecified	Fibroblast	XLRR1
EAX1		<i>Xenopus laevis</i>	Adult	Peritoneum	Epithelioid	(Arthur and Balls, 1971)
EAX2		<i>Xenopus laevis</i>	Adult	Muscle	Epithelioid	(Arthur and Balls, 1971)
EAX3		<i>Xenopus laevis</i>	Adult	Muscle	Epithelioid	(Arthur and Balls, 1971)
EAX4		<i>Xenopus laevis</i>	Adult	Liver Tumour	Epithelioid	(Arthur and Balls, 1971)
EAX5		<i>Xenopus laevis</i>	Adult	Liver & kidney	Epithelioid	(Arthur and Balls, 1971)
EAX6		<i>Xenopus laevis</i>	Adult	Liver	Epithelioid	(Arthur and Balls, 1971)
EAX7		<i>Xenopus laevis</i>	Adult	Spleen Tumour	Epithelioid	(Arthur and Balls, 1971)
EAX8		<i>Xenopus laevis</i>	Adult	Muscle	Epithelioid	(Balls and Worley, 1973)
f/k		<i>Xenopus laevis</i>	Adult	Thymic Tumour	Lymphocytoid	(Robert and Cohen, 1998)
ff-2		<i>Xenopus laevis</i>	Adult	Thymic Tumour	Lymphocytoid	(Robert et al., 1994)
ff-2.64		<i>Xenopus laevis</i>	Adult	Thymic Tumour	Lymphocytoid	(Robert et al., 1994)
KRA6		<i>Xenopus laevis</i>	Adult	Kidney	Epithelioid	(Arthur and Balls, 1971)
KRA8		<i>Xenopus laevis</i>	Adult	Liver	Epithelioid	(Arthur and Balls, 1971)
MAR1		<i>Xenopus laevis</i>	Adult	Thymic Tumour	Lymphocytoid	(Du Pasquier and Robert, 1992)
Unnamed		<i>Xenopus laevis</i>	Unspecified	Unspecified	Fibroblast	(van Zeeland et al., 1980)
Xela DS2		<i>Xenopus laevis</i>	Adult	Dorsal skin	Epithelial-like	(Bui-Marinos et al., 2020)
Xela S5F		<i>Xenopus laevis</i>	Adult	Spleen	Fibroblast-like	Todd et al. in preparation
Xela S5M		<i>Xenopus laevis</i>	Adult	Spleen	Mesothelial-like	Todd et al. in preparation
Xela VS2		<i>Xenopus laevis</i>	Adult	Ventral skin	Epithelial-like	(Bui-Marinos et al., 2020)
XF		<i>Xenopus laevis</i>	Unspecified	Unspecified	Fibroblast	(Chang and Shapiro, 1990)
XG69		<i>Xenopus laevis</i>	Adult	Retina	Epithelioid	(Gallo et al., 2022)
XL1		<i>Xenopus laevis</i>	Adult	Long bones and vertebra	Chondrocyte-like	(Conceição et al., 2013)
XL110		<i>Xenopus laevis</i>	Unspecified	Liver	Hepatocytes	(Chang and Shapiro, 1990)
XL-177		<i>Xenopus laevis</i>	Embryo	Whole	Epithelial	(Miller and Daniel, 1977)
XL177 lacZ clone 1.18	XL-177	<i>Xenopus laevis</i>	Embryo	Whole	Epithelial	(Chan and Gurdon, 1996)
XL177 lacZ clone 2.1	XL-177	<i>Xenopus laevis</i>	Embryo	Whole	Epithelial	(Chan and Gurdon, 1996)
XL177 lacZ clone 2.14	XL-177	<i>Xenopus laevis</i>	Embryo	Whole	Epithelial	(Chan and Gurdon, 1996)
XL177 lacZ clone 2.5	XL-177	<i>Xenopus laevis</i>	Embryo	Whole	Epithelial	(Chan and Gurdon, 1996)
XL2		<i>Xenopus laevis</i>	Tadpole	Unspecified	Epithelioid	(Anizet et al., 1981)
XL58		<i>Xenopus laevis</i>	Embryo	Unspecified	Unspecified	(St Germain et al., 1994)
XL58-TRE-Luc	XL58	<i>Xenopus laevis</i>	Embryo	Unspecified	Unspecified	(Sugiyama et al., 2005)
XLA		<i>Xenopus laevis</i>	Tadpole	Kidney	Epithelial	(Kanamori and Brown, 1993)
XL-B4		<i>Xenopus laevis</i>	Tadpole	Tail tip	Myoblastic	(Tamura et al., 2015)
XLg00		<i>Xenopus laevis</i>	Tadpole	Tail tip	Vascular endothelial	(Mawaribuchi et al., 2008)
XLK2		<i>Xenopus laevis</i>	Unspecified	Kidney	Unspecified	(McStay and Reeder, 1990)
XLK-WG		<i>Xenopus laevis</i>	Adult	Kidney	Epithelial	(Martin et al., 1998)
XL7-15		<i>Xenopus laevis</i>	Tadpole	Tail tip	Myoblast	(Yaoita and Nakajima, 1997)
XR1		<i>Xenopus laevis</i>	Embryo	Retinal neuroepithelium	Epithelial	(Sakaguchi et al., 1989)
XR6		<i>Xenopus laevis</i>	Embryo	Retinal neuroepithelium	Epithelial	(Sakaguchi et al., 1989)
XR8		<i>Xenopus laevis</i>	Embryo	Retinal neuroepithelium	Epithelial	(Sakaguchi et al., 1989)
XR9		<i>Xenopus laevis</i>	Embryo	Retinal neuroepithelium	Epithelial	(Sakaguchi et al., 1989)
XRpe1		<i>Xenopus laevis</i>	Embryo	Retinal pigment epithelium	Epithelial	(Sakaguchi and Henderson, 1993)
XRpe2		<i>Xenopus laevis</i>	Embryo	Retinal pigment epithelium	Epithelial	(Sakaguchi and Henderson, 1993)
XRpe4		<i>Xenopus laevis</i>	Embryo	Retinal pigment epithelium	Epithelial	(Sakaguchi and Henderson, 1993)
XRpe5		<i>Xenopus laevis</i>	Embryo	Retinal pigment epithelium	Epithelial	(Sakaguchi and Henderson, 1993)
XTC-2 [BirAV5]	XTC-2	<i>Xenopus laevis</i>	Tadpole	Whole	Fibroblast-like	Cellosaurus: CVCL_C0NH
XTC-2 [BirAV5-FLBIO-xKLF9]	XTC-2	<i>Xenopus laevis</i>	Tadpole	Whole	Fibroblast-like	Cellosaurus: CVCL_C0NI
XTC-2		<i>Xenopus laevis</i>	Tadpole	Whole	Fibroblast-like	(Pudney et al., 1973)
XTC-GTX-11	XTC-2	<i>Xenopus laevis</i>	Tadpole	Whole	Fibroblast-like	(van den Eijnden-Van Raaij et al., 1990)
XTH-2		<i>Xenopus laevis</i>	Tadpole	Heart	Endothelial	Schlage et al. (1981)

XTY		<i>Xenopus laevis</i>	Adult	Subcutaneous tumour	Fibroblast-like	(Fukui et al., 1992)
LM6		<i>Xenopus laevis</i> x <i>Xenopus muelleri</i>	Tadpole	Whole (without tail, intestines)	Fibroblastoid	(Plytycz et al., 1988)
91.1.F1		<i>Xenopus tropicalis</i>	Tadpole	Hindlimb	Epithelioid	(Sinzelle et al., 2012)
XTN-10		<i>Xenopus tropicalis</i>	Embryo	Whole	Unknown	(Gorbsky et al., 2022)
XTN-12		<i>Xenopus tropicalis</i>	Embryo	Whole	Unknown	(Gorbsky et al., 2022)
XTN-6		<i>Xenopus tropicalis</i>	Embryo	Whole	Unknown	(Gorbsky et al., 2022)
XTN-8		<i>Xenopus tropicalis</i>	Embryo	Whole	Unknown	(Gorbsky et al., 2022)
Speedy	91.1.F1	<i>Xenopus tropicalis</i>	Tadpole	Limb bud	Fibroblast	(Sinzelle et al., 2012)

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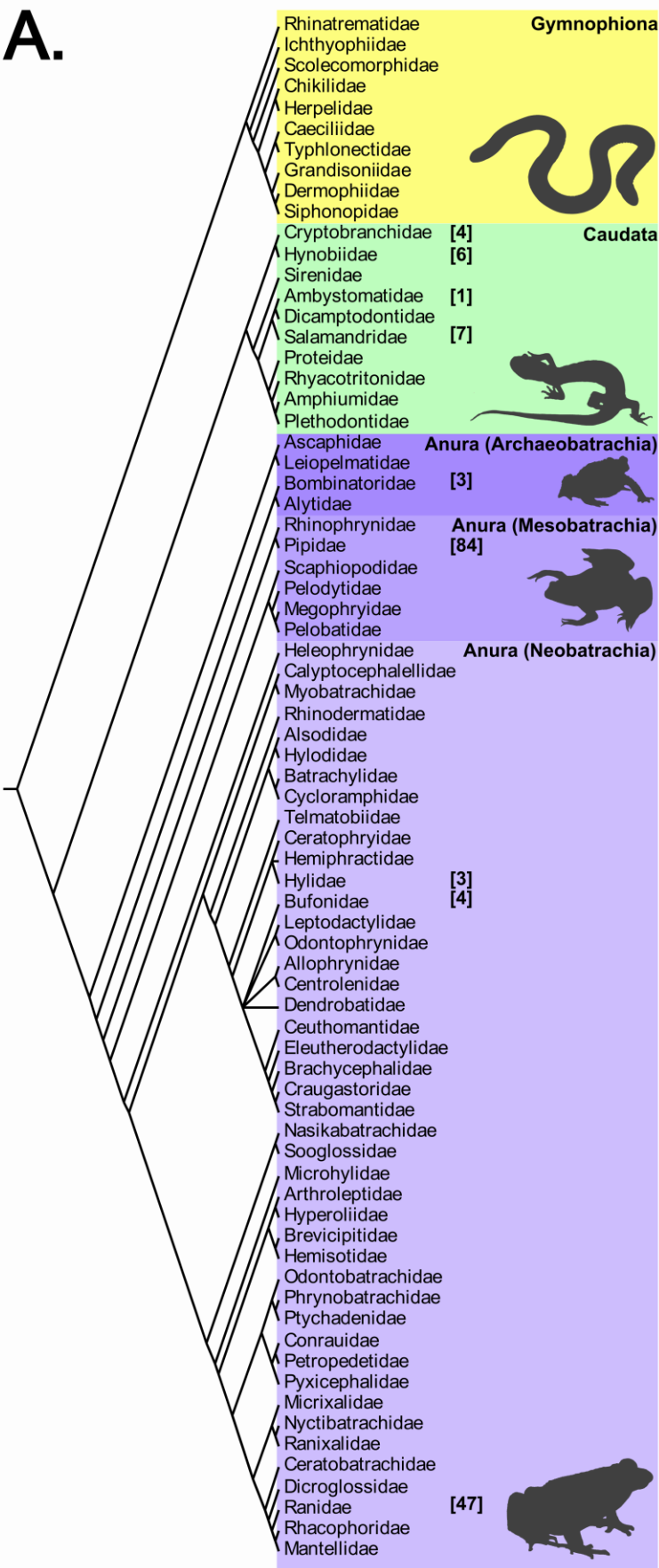
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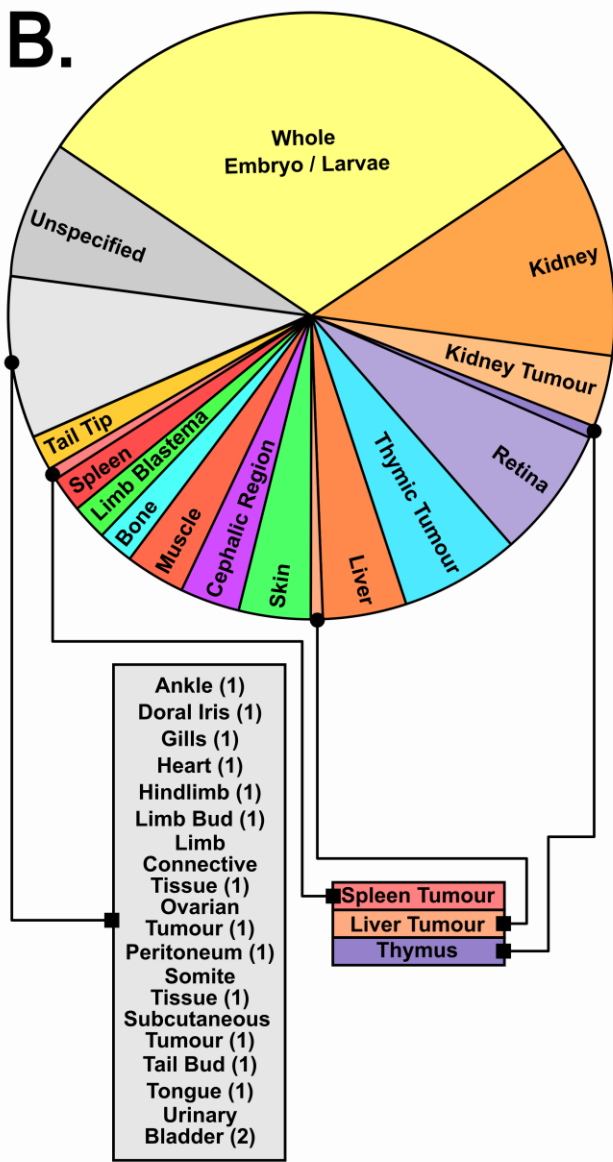
1582 **Figure 1. Timeline of amphibian cell line development and select immunological milestones.**

1583 Since the development of the first reported amphibian cell line in 1964, over 150 distinct
1584 amphibian cell lines have been developed. The number of novel anuran and caudatan cell lines
1585 reported, as well as the cumulative number of amphibian cell lines, are given for each year.
1586 Amphibian cell lines have been used to study host-pathogen interactions since they were first
1587 developed and continue to support our understanding of amphibian immune responses today.
1588 Abbreviations: *Bd*, *Batrachochytrium dendrobatidis*; FV3, frog virus 3; TNF, tumour necrosis
1589 factor.

A.



B.



C.

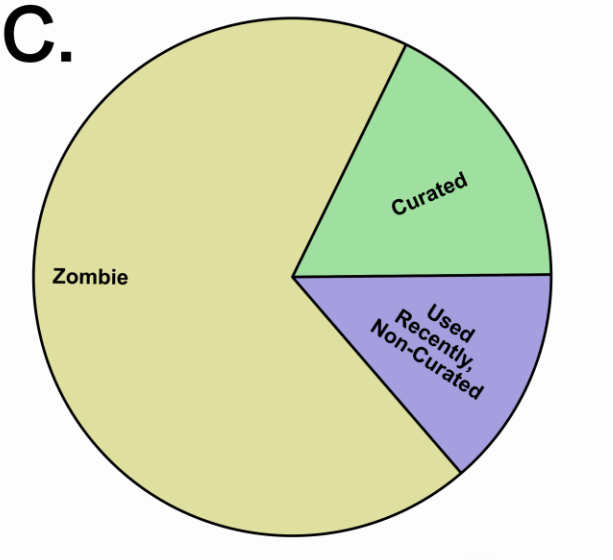


Figure 2. The current state of the amphibian invitrome. **A.** A cladogram depicting the evolutionary relationships between extant amphibian orders and families, adapted from AmphibiaWeb's Family Phylogeny (AmphibiaWeb, 2022). Bolded numbers in square brackets indicate the number of reported cell lines developed from members of each family, if any. **B.** A pie chart summarizing the tissue origins of the reported amphibian cell lines. **C.** A pie chart summarizing the storage and availability status of the reported amphibian cell lines into cell lines in the curated invitrome (cell lines held in collections of formal agencies), those that have been recently used in the literature but are not curated and are likely in the informally shared invitrome (informally shared between researchers), and those that are likely in the zombie invitrome (cell lines that have been described in publications but do not appear to be used or stored any longer).

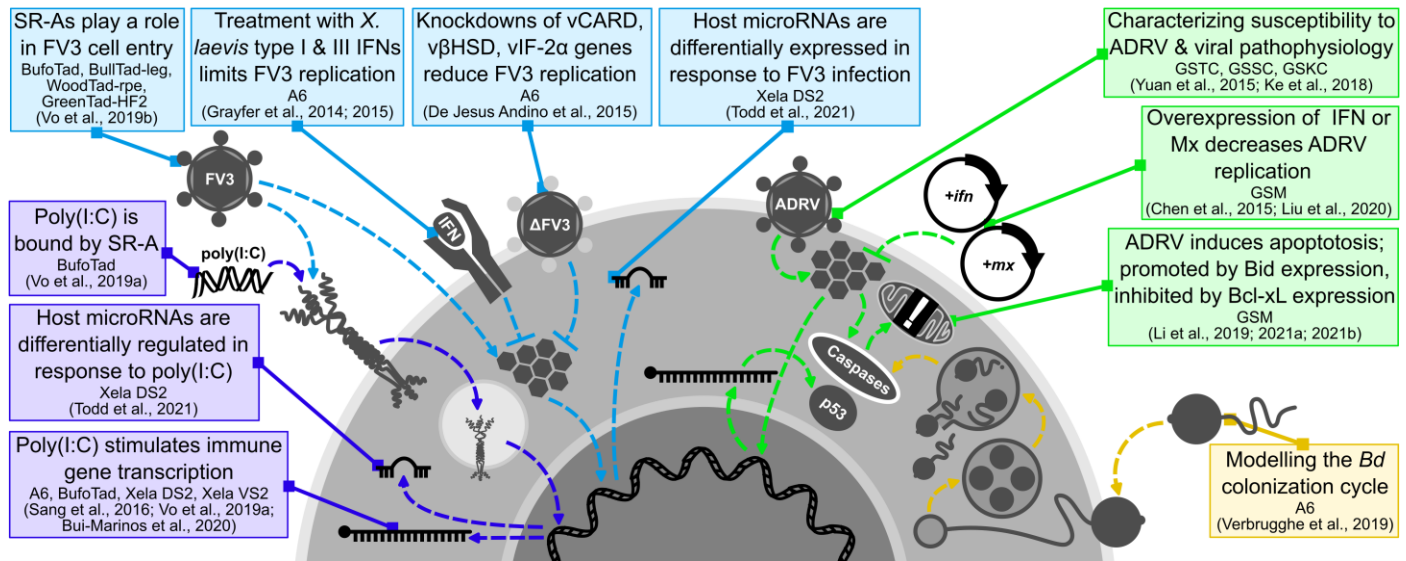


Figure 3. Recent advances in amphibian immunology made using amphibian cell lines. In the past decade (2012 – 2022), immunological studies relying on amphibian cell lines have focused on monitoring responses to poly(I:C) (violet, described in Section 3.2.1), FV3 (blue, described in Section 3.3.1.1), ADRV (green, described in Section 3.3.1.2), and *Bd* (yellow, described in Section 3.3.2). While A6 continues to be the most widely used amphibian cell line, several recently developed cell lines from varied species and tissue origins have made major contributions. Abbreviations: ADRV, *Andrias davidianus* ranavirus; Bcl-xL, B-cell lymphoma-extra large; *Bd*, *Batrachochytrium dendrobatidis*; Bid, BH3-interacting domain death agonist; SR-A, Class A Scavenger Receptor; FV3, frog virus 3; IFN, interferon; poly(I:C), polyinosinic:polycytidylic acid; vβHSD, viral β-hydroxysteroid dehydrogenase; vCARD, viral caspase activation and recruitment domain-containing protein; vIF-2α, viral eukaryotic translation initiation factor 2 subunit 1.

Supplementary Material 1. Literature Search Methodology

The PubMed database was searched on April 9, 2022 using the following search string: “(((“in vitro”[Title/Abstract] OR in-vitro[Title/Abstract] OR “ex vivo”[Title/Abstract] OR ex-vivo[Title/Abstract] OR “non lethal”[Title/Abstract]) OR (“In Vitro Techniques”[Mesh])) AND (((“Cell Line”[Mesh]) OR (“cell line”[Title/Abstract] OR “cell culture”[Title/Abstract] OR “cultured cells” OR “tissue explant*” OR “cell source*”)) OR (“Cells, Cultured”[MeSH])) AND ((amphibian*[Title/Abstract] OR frog*[Title/Abstract] OR anura[Title/Abstract] OR urodel*[Title/Abstract] OR salamand*[Title/Abstract] OR apoda[Title/Abstract] OR caecilia*[Title/Abstract]) OR (“amphibians”[MeSH Major Topic]))”. This search resulted in 1,224 non-redundant articles which were then imported into Covidence (Covidence systematic review software, Veritas Health Innovation, Melbourne, Australia. Available at www.covidence.org.) to be screened for relevance. Articles were deemed relevant if at least one of the following conditions were met: (1) The study generated a new amphibian cell line, (2) the study used an amphibian cell line to conduct research related to immunology, or (3) the study used primary cell cultures or tissue explants to conduct research related to immunology. After irrelevant articles were filtered out, 68 relevant articles remained and full-texts were downloaded from Covidence for in-depth review. In addition, hand searching was performed as required.

Supplementary Table 1. Amphibian cell line information.

Cell line	Parent	Reference	Year Reported	Cellosaurus Entry	Species	Sex	Stage	Tissue of origin	Transformation / Immortalization Method	Morphology	Cell Bank Availability	Commercial Provider Availability	Published on within ≤ 5 years?
ACT1 (aka AL1)		(Roy et al., 2000)	2000	None	<i>Ambystoma mexicanum</i>	Unspecified	Larva	Limb connective tissue	Spontaneous	Fibroblast			No
BA 68.1		(Freed et al., 1969)	1969	CVCL_LN07	<i>Anaxyrus americanus</i>	Unspecified	Embryo	Unspecified	Unspecified	Epithelioid			No
BufoTad		(Vo et al., 2019a)	2019	None	<i>Anaxyrus americanus</i>	Unspecified	Tadpole	Whole	Spontaneous	Epithelial/Endothelial			Yes
GSKC		(Yuan et al., 2015)	2015	CVCL_1G56	<i>Andrias davidianus</i>	Unspecified	Adult	Kidney	Spontaneous	Epithelial-like			No
GSM		(Chen et al., 2015)	2015	None	<i>Andrias davidianus</i>	Unspecified	Unspecified	Muscle	Unspecified	Spindle-Shaped			Yes
GSSC		(Yuan et al., 2015)	2015	CVCL_1G57	<i>Andrias davidianus</i>	Unspecified	Adult	Spleen	Spontaneous	Epithelial-like			No
GSTC		(Yuan et al., 2015)	2015	CVCL_1G58	<i>Andrias davidianus</i>	Unspecified	Adult	Thymus	Spontaneous	Epithelial-like			Yes
Unnamed		(Xiang et al., 2012)	2012	None	<i>Bombina maxima</i>	Unspecified	Tadpole	Whole Skin (without tail)	Transformation with SV40 Large T antigen	Fibroblast			No
Bok-2		(Park et al., 2000)	2000	None	<i>Bombina orientalis</i>	Unspecified	Embryo	Tail bud	Spontaneous	Ameboid/Phagocytic			No
Bor II		(Ellinger et al., 1983)	1983	CVCL_T699	<i>Bombina orientalis</i>	Unspecified	Embryo	Whole	Spontaneous	Epithelioid/Fibroblastoid			No
TB-6c		(Handler et al., 1979)	1979	CVCL_M351	<i>Bufo marinus</i>	Unspecified	Unspecified	Urinary Bladder	Spontaneous	Epithelial			No
TBM-54		(Handler et al., 1979)	1979	CVCL_5654	<i>Bufo marinus</i>	Unspecified	Unspecified	Urinary Bladder	Spontaneous	Epithelial	ATCC Cat#: CRL-2051		No
CPN4B		None	1995	CVCL_7253	<i>Cynops pyrrhogaster</i>	Unspecified	Embryo	Whole	Spontaneous	Epithelial-like	RCB Cat#: RCB1690		No
FUHEN		(Sugimoto, 2015)	2015	CVCL_4U91	<i>Dryophytes japonicus</i>	Unspecified	Adult	Bone marrow	Spontaneous	Thrombocytic			No
HNS1A		None	1988	CVCL_6650	<i>Hynobius nebulosus</i>	Unspecified	Embryo	Whole	Spontaneous	Epithelioid	RCB Cat#: RCB0591		No
HNS2		None	1988	CVCL_6651	<i>Hynobius nebulosus</i>	Unspecified	Embryo	Whole	Spontaneous	Epithelioid	RCB Cat#: RCB0592		No
HNS3		None	1988	CVCL_6652	<i>Hynobius nebulosus</i>	Unspecified	Embryo	Whole	Spontaneous	Fibroblastoid	RCB Cat#: RCB0593		No
HNS4		None	1988	CVCL_6653	<i>Hynobius nebulosus</i>	Unspecified	Embryo	Whole	Spontaneous	Epithelioid	RCB Cat#: RCB0594		No
HNS5		None	1988	CVCL_6654	<i>Hynobius nebulosus</i>	Unspecified	Embryo	Whole	Spontaneous	Fibroblastoid	RCB Cat#: RCB0595		No
HTUD1		None	1991	CVCL_L695	<i>Hynobius tokyoensis</i>	Female	Embryo	Whole	Spontaneous	Epithelioid	RCB Cat#: RCB0713		No
A1		(Ferretti and Brockes, 1988)	1988	CVCL_B7RL	<i>Notophthalmus viridescens</i>	Unspecified	Adult	Ankle	Spontaneous	Fibroblastoid			Yes
TV1		(Reese et al., 1976)	1976	CVCL_B7QJ	<i>Notophthalmus viridescens</i>	Unspecified	Adult	Dorsal iris	Spontaneous	Epithelial			No
BIH1 / B1H1		(Ferretti and Brockes, 1988)	1988	CVCL_B7RB	<i>Notophthalmus viridescens</i>	Unspecified	Adult	Hind Limb Blastema	Spontaneous	Fibroblastoid			No
NvSV40-1		(Washabaugh, 1994)	1994	None	<i>Notophthalmus viridescens</i>	Unspecified	Unspecified	Limb Blastema	Transformation with SV40 Large T antigen	Fibroblastoid			No

NvSV40-B3		(Washabaugh, 1994)	1994	None	<i>Notophthalmus viridescens</i>	Unspecified	Unspecified	Limb Blastema	Transformation with SV40 Large T antigen	Fibroblastoid			No
TH4B		(Ferretti and Brockes, 1988)	1988	CVCL_B7RC	<i>Notophthalmus viridescens</i>	Unspecified	Adult	Thigh muscle	Spontaneous	Mesenchymal			No
A-11		(Rafferty, 1969)	1969	None	<i>Pseudacris crucifer</i>	Unspecified	Hatching embryo	Whole	Spontaneous	Mixed			No
A-4		(Rafferty, 1969)	1969	None	<i>Pseudacris triseriata</i>	Unspecified	Hatching embryo	Whole	Spontaneous	Mixed			No
LAH3		(Okumoto, 2001)	2001	CVCL_6668	<i>Rana brevipoda</i>	Unspecified	Froglet	Dorsal skin	Spontaneous	Epithelial-like melanophores	RCB Cat#: RCB1734		No
HO(6)		None		CVCL_Y444	<i>Rana catesbeiana</i>	Unspecified	Unspecified	Unspecified	Spontaneous	Unspecified	ATCC Cat#: CRL-6267 (Discontinued)		No
BullTad-leg		(Vo et al., 2019b)	2019	None	<i>Rana catesbeiana</i>	Unspecified	Tadpole	Unspecified	Unspecified	Fibroblastoid			Yes
FT		(Wolf and Quimby, 1964)	1964	CVCL_4203	<i>Rana catesbeiana</i>	Female	Adult	Tongue	Spontaneous	Fibroblast	ATCC Cat#: CCL-41, ECACC Cat#: 90102525		No
A-19		(Rafferty, 1969)	1969	None	<i>Rana clamitans</i>	Unspecified	Hatching embryo	Whole	Spontaneous	Fibroblast			No
A-20		(Rafferty, 1969)	1969	None	<i>Rana clamitans</i>	Unspecified	Hatching embryo	Whole	Spontaneous	Fibroblast			No
A-5		(Rafferty, 1969)	1969	None	<i>Rana clamitans</i>	Unspecified	Hatching embryo	Whole	Spontaneous	Fibroblast			No
GreenTad-gill		(Vo et al., 2019c)	2019	CVCL_A0WG	<i>Rana clamitans</i>	Unspecified	Tadpole	Gills	Spontaneous	Epithelial-like			Yes
GreenTad-HE4		(Vo et al., 2019c)	2019	CVCL_A0WF	<i>Rana clamitans</i>	Unspecified	Tadpole	Cephalic region	Spontaneous	Epithelial-like			Yes
GreenTad-HF1		(Vo et al., 2019c)	2019	CVCL_A0WC	<i>Rana clamitans</i>	Unspecified	Tadpole	Cephalic region	Spontaneous	Fibroblast-like			Yes
GreenTad-HF2		(Vo et al., 2019b)	2019	CVCL_A0WD	<i>Rana clamitans</i>	Unspecified	Tadpole	Cephalic region	Spontaneous	Fibroblast-like			Yes
GreenTad-HF3		(Vo et al., 2019c)	2019	CVCL_A0WE	<i>Rana clamitans</i>	Unspecified	Tadpole	Cephalic region	Spontaneous	Fibroblast-like			Yes
LAH1		(Okumoto, 2001)	2001	CVCL_6666	<i>Rana nigromaculata</i>	Unspecified	Froglet	Dorsal skin	Spontaneous	Mixed melanophores	RCB Cat#: RCB1733 (Discontinued)		No
LAH2		(Okumoto, 2001)	2001	CVCL_6667	<i>Rana nigromaculata</i> (albino)	Unspecified	Froglet	Dorsal skin	Spontaneous	Mixed melanophores			No
WERPa		(Rafferty, 1965)	1965	None	<i>Rana palustris</i>	Unspecified	Embryo	Whole	Spontaneous	Epithelioid			No, cell line collapsed
3AKRP		(Gravell, 1971)	1971	CVCL_B7S9	<i>Rana pipiens</i>	Unspecified	Adult	Kidney	Unspecified	Unspecified			No
A-2		(Rafferty, 1969)	1969	None	<i>Rana pipiens</i>	Unspecified	Hatching embryo	Whole	Spontaneous	Epithelioid			No
A-3		(Rafferty, 1969)	1969	None	<i>Rana pipiens</i>	Female	Adult	Lucke tumor	Spontaneous	Epithelioid			No
DRP 153	ICR-2A	(Rosenstein, 1989)	1989	CVCL_T701	<i>Rana pipiens</i>	Unspecified	Embryo, Androgenetic Haploid	Whole	Spontaneous	Fibroblast			No
DRP 36	ICR-2A	(Rosenstein and Chao, 1985)	1985	CVCL_T700	<i>Rana pipiens</i>	Unspecified	Embryo, Androgenetic Haploid	Whole	Spontaneous	Fibroblast			No

ICR B20	ICR-2A	(Freed, 1972)	1972	CVCL_5G40	<i>Rana pipiens</i>	Unspecified	Embryo, Androgenetic Haploid	Whole	Spontaneous	Fibroblast			No
ICR B9	ICR-2A	(Freed, 1972)	1972	CVCL_B0V7	<i>Rana pipiens</i>	Unspecified	Embryo, Androgenetic Haploid	Whole	Spontaneous	Fibroblast			No
ICR-134 / RPH 67.134		(Freed et al., 1969)	1969	CVCL_6408	<i>Rana pipiens</i>	Unspecified	Embryo	Whole	Spontaneous	Fibroblastoid	ATCC Cat#: CCL-128		No
ICR-2A1	ICR-2A	(Freed, 1972)	1972	CVCL_B0V5	<i>Rana pipiens</i>	Unspecified	Embryo, Androgenetic Haploid	Whole	Spontaneous	Fibroblast			No
ICR-2A2	ICR-2A	(Freed, 1972)	1972	CVCL_B0V6	<i>Rana pipiens</i>	Unspecified	Embryo, Androgenetic Haploid	Whole	Spontaneous	Fibroblast			No
PNKT-3		(Tweedell and Williams, 1976)	1976	CVCL_T685	<i>Rana pipiens</i>	Unspecified	Embryo	Pronephric tumour	Lucke herpesvirus	Mixed epithelioid/fibroblast			No
PNKT-4		(Tweedell and Williams, 1976)	1976	CVCL_T687	<i>Rana pipiens</i>	Unspecified	Embryo	Pronephric tumour	Lucke herpesvirus	Mixed epithelioid/fibroblast			No
PRKT		(Tweedell and Williams, 1976)	1976	CVCL_T686	<i>Rana pipiens</i>	Unspecified	Embryo	Pronephric tumour	Lucke herpesvirus	Mixed epithelioid/fibroblast			No
RPD 67.7		(Freed et al., 1969)	1969	CVCL_LN14	<i>Rana pipiens</i>	Unspecified	Embryo	Unspecified	Spontaneous	Mixed epithelioid/fibroblast			No
RPH 67.194		(Freed et al., 1969)	1969	CVCL_LN06	<i>Rana pipiens</i>	Unspecified	Unspecified	Somite tissue	Spontaneous	Mixed			No
WERPi		(Rafferty, 1965)	1965	None	<i>Rana pipiens</i>	Unspecified	Embryo	Whole	Spontaneous	Epithelioid			No
LC-1		(Rafferty, 1965)	1965	None	<i>Rana pipiens</i>	Unspecified	Adult	Lucké Renal Carcinoma	Spontaneous	Epithelioid			No
ICR-2A / RPH 68.2A		((Freed and Mezger-Freed, 1970)	1970	CVCL_4345	<i>Rana pipiens</i>	Unspecified	Embryo, Androgenetic Haploid	Whole	Spontaneous	Fibroblast	ATCC Cat#: CCL-145, ECACC Cat#: 89072615, IZSLER Cat#: BS CL 191		No
PNKT-4B		(Tweedell, 1978)	1978	CVCL_T688	<i>Rana pipiens</i>	Unspecified	Embryo	Pronephric tumour	Lucke herpesvirus	Unspecified			No
RPH 67.205		(Freed and Mezger-Freed, 1970)	1970	CVCL_6725	<i>Rana pipiens</i>	Unspecified	Embryo, Androgenetic Haploid	Whole	Spontaneous	Epithelial-like			No
RPKID		(Tweedell and Lazar, 1992)	1992	CVCL_T698	<i>Rana pipiens</i>	Unspecified	Unspecified	Kidney	Spontaneous	Unspecified			No
WMPa		(Wong and Tweedell, 1974)	1974	CVCL_T689	<i>Rana pipiens</i>	Unspecified	Embryo	Kidney	Spontaneous	Mixed epithelioid			No
A-1		(Rafferty, 1969)	1969	None	<i>Rana sylvatica</i>	Unspecified	Large embryo	Kidney	Spontaneous	Epithelioid			No
A-21		(Rafferty, 1969)	1969	None	<i>Rana sylvatica</i>	Unspecified	Hatching embryo	Whole	Spontaneous	Epithelioid			No

A-22		(Rafferty, 1969)	1969	None	<i>Rana sylvatica</i>	Unspecified	Hatching embryo	Whole	Spontaneous	Epithelioid			No
A-23F		(Rafferty, 1969)	1969	None	<i>Rana sylvatica</i>	Unspecified	Hatching embryo	Whole	Spontaneous	Epithelioid			No
A-23R		(Rafferty, 1969)	1969	None	<i>Rana sylvatica</i>	Unspecified	Hatching embryo	Whole	Spontaneous	Epithelioid			No
KERS		(Rafferty, 1969)	1965	None	<i>Rana sylvatica</i>	Unspecified	Embryo	Kidney	Spontaneous	Epithelioid			No
WERS		(Rafferty, 1965)	1965	None	<i>Rana sylvatica</i>	Unspecified	Embryo	Whole	Spontaneous	Epithelioid			No
WoodTad-Bone		(Vo et al., 2019c)	2019	CVCL_A0WI	<i>Rana sylvatica</i>	Unspecified	Tadpole	Hind leg bone	Spontaneous	Fibroblast-like			Yes
WoodTad-HE1		(Vo et al., 2019c)	2019	CVCL_A0WH	<i>Rana sylvatica</i>	Unspecified	Tadpole	Cephalic region	Spontaneous	Epithelial-like			Yes
WoodTad-rpe		(Vo et al., 2019b)	2019	CVCL_A0WJ	<i>Rana sylvatica</i>	Unspecified	Tadpole	Retinal tissue	Spontaneous	Epithelial-like			Yes
XB691		(Picard et al., 1983)	1983	CVCL_C0Q2	<i>Xenopus borealis</i>	Unspecified	Tadpole	Whole	Spontaneous	Epithelioid			No
XB693		(Picard et al., 1983)	1983	CVCL_C0Q3	<i>Xenopus borealis</i>	Unspecified	Tadpole	Whole	Spontaneous	Epithelioid			No
XB693T	XB693	(Picard et al., 1983)	1983	CVCL_C0Q4	<i>Xenopus borealis</i>	Unspecified	Tadpole	Whole	Treatment with 1-Methyl-3-nitro-1-nitrosoguanidine	Epithelioid			No
XB694		(Picard et al., 1983)	1983	CVCL_UJ64	<i>Xenopus borealis</i>	Unspecified	Tadpole	Whole	Spontaneous	Epithelioid			No
S3 / S3-1		(Cohen et al., 2000)	2000	CVCL_GY00	<i>Xenopus laevis</i>	Unspecified	Embryo	Unspecified	Spontaneous	Unspecified			No
15/0		(Robert et al., 1994)	1994	None	<i>Xenopus laevis</i> (LG-15 strain)	Unspecified	Adult	Thymic Tumour	Spontaneous	Lymphocytoid	XLRR1		Yes
15/0 (GFP+)	15/0	None		None	<i>Xenopus laevis</i> (LG-15 strain)	Unspecified	Adult	Thymic Tumour	Transformed to express recombinant Green Fluorescent Protein	Lymphocytoid	XLRR1		No
15/0 (a haplotype class Ia+)	15/0	None		None	<i>Xenopus laevis</i> (LG-15 strain)	Unspecified	Adult	Thymic Tumour	Transformed to express recombinant a haplotype MHC class Ia	Lymphocytoid	XLRR1		No
15/0 (b2m knockdown)	15/0	None		None	<i>Xenopus laevis</i> (LG-15 strain)	Unspecified	Adult	Thymic Tumour	Transformed to knockdown Beta-2 microglobulin	Lymphocytoid	XLRR1		No
15/40		(Robert et al., 1994)	1994	None	<i>Xenopus laevis</i>	Unspecified	Adult	Thymic Tumour	Spontaneous	Lymphocytoid	XLRR1		No
A-24		(Rafferty, 1969)	1969	None	<i>Xenopus laevis</i>	Female	Adult	Ovarian tumour	Spontaneous	Epithelioid			No
A6		(Rafferty, 1969)	1969	CVCL_4101	<i>Xenopus laevis</i>	Male	Adult	Kidney	Spontaneous	Epithelioid	ATCC Cat#: CCL-102, ECACC Cat#: 89072613, RCB Cat#: RCB0772, XLRR1		Yes
A6/C1	A6-2F3	(Verrey et al., 1987)	1987	CVCL_B7ED	<i>Xenopus laevis</i>	Male	Adult	Kidney	Spontaneous	Epithelioid			No
A6-2F3	A6	(Wills et al., 1992)	1992	CVCL_F714	<i>Xenopus laevis</i>	Male	Adult	Kidney	Spontaneous	Epithelioid			No
A6-KR	A6	(Ellison et al., 1985)	1985	CVCL_VQ55	<i>Xenopus laevis</i>	Male	Adult	Kidney	Spontaneous	Epithelioid			No
A-7		(Rafferty, 1969)	1969	None	<i>Xenopus laevis</i>	Male	Adult	Kidney	Spontaneous	Epithelioid			No

A8 2k		(Chinchar and Sinclair, 1978)	1978	CVCL_C0YN	<i>Xenopus laevis</i>	Female	Adult	Liver	Ethyl methanesulfonate	Epithelioid			No
A8		(Rafferty, 1969)	1969	CVCL_4564	<i>Xenopus laevis</i>	Female	Adult	Liver	Spontaneous	Epithelioid	RCB Cat#: RCB0773		No
A8W243	A8	(GRIGGS and BENDER, 1972)	1972	None	<i>Xenopus laevis</i>	Female	Adult	Liver	Spontaneous	Epithelioid			No
ACTK1		None		CVCL_BS99	<i>Xenopus laevis</i>	Unspecified	Unspecified	Kidney	Unknown	Unknown	KCB Cat#: KCB 82004YJ		No
B3.2		(Seiler-Tuyns et al., 1988)	1988	CVCL_T690	<i>Xenopus laevis</i>	Unspecified	Unspecified	Kidney	Spontaneous	Unknown			No
B3.2(tTA)1	B3.2	(Camacho-Vanegas et al., 1998)	1998	CVCL_6E63	<i>Xenopus laevis</i>	Unspecified	Unspecified	Kidney	pUHD15.1 vector carrying tTA gene	Unknown			No
B3B7	MAR1	(du Pasquier and Robert, 1992)	1992	None	<i>Xenopus laevis</i> (F strain)	Male	Adult	Thymic Tumour	Spontaneous	Lymphocytoid	XLRR1		No
B3D3	MAR1	(du Pasquier and Robert, 1992)	1992	None	<i>Xenopus laevis</i>	Male	Adult	Thymic Tumour	Spontaneous	Lymphocytoid			No
DWJ		None		None	<i>Xenopus laevis</i> (J strain)	Unspecified	Unspecified	Unspecified	Unspecified	Fibroblast	XLRR1		No
DW15		None		None	<i>Xenopus laevis</i> (LG-15 strain)	Unspecified	Unspecified	Unspecified	Unspecified	Fibroblast	XLRR1		No
EAX1		(Arthur and Balls, 1971)	1971	None	<i>Xenopus laevis</i>	Unspecified	Adult	Peritoneum	Spontaneous	Epithelioid			No
EAX2		(Arthur and Balls, 1971)	1971	None	<i>Xenopus laevis</i>	Unspecified	Adult	Muscle	Spontaneous	Epithelioid			No
EAX3		(Arthur and Balls, 1971)	1971	None	<i>Xenopus laevis</i>	Unspecified	Adult	Muscle	Spontaneous	Epithelioid			No
EAX4		(Arthur and Balls, 1971)	1971	None	<i>Xenopus laevis</i>	Unspecified	Adult	Liver Tumour	Spontaneous	Epithelioid			No
EAX5		(Arthur and Balls, 1971)	1971	None	<i>Xenopus laevis</i>	Unspecified	Adult	Liver & kidney	Spontaneous	Epithelioid			No
EAX6		(Arthur and Balls, 1971)	1971	None	<i>Xenopus laevis</i>	Unspecified	Adult	Liver	Spontaneous	Epithelioid			No
EAX7		(Arthur and Balls, 1971)	1971	None	<i>Xenopus laevis</i>	Unspecified	Adult	Spleen Tumour	Spontaneous	Epithelioid			No
EAX8		(Balls and Worley, 1973)	1973	None	<i>Xenopus laevis</i>	Unspecified	Adult	Muscle	Spontaneous	Epithelioid			No
f/k		(Robert and Cohen, 1998)	1998	None	<i>Xenopus laevis</i>	Male	Adult	Thymic Tumour	Spontaneous	Lymphocytoid			No
ff-2		(Robert et al., 1994)	1994	None	<i>Xenopus laevis</i> (F strain)	Male	Adult	Thymic Tumour	Spontaneous	Lymphocytoid	XLRR1		Yes
ff-2.64		(Robert et al., 1994)	1994	None	<i>Xenopus laevis</i>	Male	Adult	Thymic Tumour	Spontaneous	Lymphocytoid			No
KRA6		(Arthur and Balls, 1971)	1971	None	<i>Xenopus laevis</i>	Unspecified	Adult	Kidney	Spontaneous	Epithelioid			No

KRA8		(Arthur and Balls, 1971)	1971	None	<i>Xenopus laevis</i>	Unspecified	Adult	Liver	Spontaneous	Epithelioid			No
MAR1		(du Pasquier and Robert, 1992)	1992	None	<i>Xenopus laevis</i>	Male	Adult	Thymic Tumour	Spontaneous	Lymphocytoid			No
Unnamed		(van Zeeland et al., 1980)	1980	None	<i>Xenopus laevis</i>	Unspecified	Unspecified	Unspecified	Unspecified	Fibroblast			No
Xela DS2		(Bui-Marinos et al., 2020)	2020	CVCL_C0XN	<i>Xenopus laevis</i>	Female	Adult	Dorsal skin	Spontaneous	Epithelial-like			Yes
Xela S5F		Todd et al. in preparation		None	<i>Xenopus laevis</i>	Female	Adult	Spleen	Spontaneous	Fibroblast-like			Yes
Xela S5M		Todd et al. in preparation		None	<i>Xenopus laevis</i>	Female	Adult	Spleen	Spontaneous	Mesothelial-like			Yes
Xela VS2		(Bui-Marinos et al., 2020)	2020	CVCL_C0XP	<i>Xenopus laevis</i>	Female	Adult	Ventral skin	Spontaneous	Epithelial-like			Yes
XF		(Chang and Shapiro, 1990)	1990	CVCL_6E64	<i>Xenopus laevis</i>	Unspecified	Unspecified	Unspecified	Spontaneous	Fibroblast			No
XG69		(Gallo et al., 2022)	2022	CVCL_C0XL	<i>Xenopus laevis</i>	Unspecified	Adult	Retina	Spontaneous	Epithelioid			Yes
<i>XL1</i>		(Conceição et al., 2013)	2013	CVCL_6E60	<i>Xenopus laevis</i>	Unspecified	Adult	Long bones and vertebra	Spontaneous	Chondrocyte-like			No
<i>XL110</i>		(Chang and Shapiro, 1990)	1990	CVCL_T702	<i>Xenopus laevis</i>	Unspecified	Unspecified	Liver	Spontaneous	Hepatocytes			No
XL-177		(Miller and Daniel, 1977)	1977	CVCL_T684	<i>Xenopus laevis</i>	Unspecified	Embryo	Whole	Spontaneous	Epithelial			No
XL177 lacZ clone 1.18	XL-177	(Chan and Gurdon, 1996)	1996	CVCL_C0N8	<i>Xenopus laevis</i>	Unspecified	Embryo	Whole	Transfected with <i>Escherichia coli</i> lacZ, <i>Klebsiella pneumoniae</i> transposon Tn5 neo	Epithelial			No
XL177 lacZ clone 2.1	XL-177	(Chan and Gurdon, 1996)	1996	CVCL_C0N9	<i>Xenopus laevis</i>	Unspecified	Embryo	Whole	Transfected with <i>Escherichia coli</i> lacZ, <i>Klebsiella pneumoniae</i> transposon Tn5 neo	Epithelial			No
XL177 lacZ clone 2.14	XL-177	(Chan and Gurdon, 1996)	1996	CVCL_C0NA	<i>Xenopus laevis</i>	Unspecified	Embryo	Whole	Transfected with <i>Escherichia coli</i> lacZ, <i>Klebsiella pneumoniae</i> transposon Tn5 neo	Epithelial			No
XL177 lacZ clone 2.5	XL-177	(Chan and Gurdon, 1996)	1996	CVCL_C0NB	<i>Xenopus laevis</i>	Unspecified	Embryo	Whole	Transfected with <i>Escherichia coli</i> lacZ, <i>Klebsiella pneumoniae</i> transposon Tn5 neo	Epithelial			No
XL2		(Anizet et al., 1981)	1981	CVCL_6743	<i>Xenopus laevis</i>	Unspecified	Tadpole	Unspecified	Spontaneous	Epithelioid			No
XL58		(St Germain et al., 1994)	1994	CVCL-C0NC	<i>Xenopus laevis</i>	Unspecified	Embryo	Unspecified	Unspecified	Unspecified			No
XL58-TRE-Luc	XL58	(Sugiyama et al., 2005)	2005	CVCL-C0ND	<i>Xenopus laevis</i>	Unspecified	Embryo	Unspecified	Transduced with the LV-TRE-Luc lentiviral vector, containing the firely luciferase gene downstream from <i>X. laevis</i> thyroid hormone response elements and the SV40 promoter.	Unspecified			No
XLA		(Kanamori and Brown, 1993)	1993	None	<i>Xenopus laevis</i>	Unspecified	Tadpole	Kidney	Spontaneous	Epithelial			No
XL-B4		(Tamura et al., 2015)	2015	None	<i>Xenopus laevis</i>	Male	Tadpole	Tail tip	Spontaneous	Myoblastic			No

XLgoo		(Mawaribuchi et al., 2008)	2008	None	<i>Xenopus laevis</i>	Unspecified	Tadpole	Tail tip	Spontaneous	Vascular endothelial			No
XLK2		(McStay and Reeder, 1990)	1990	CVCL_H009	<i>Xenopus laevis</i>	Unspecified	Unspecified	Kidney	Unspecified	Unspecified			No
XLK-WG		(Martin et al., 1998)	1998	CVCL_5655	<i>Xenopus laevis</i>	Female	Adult	Kidney	Spontaneous	Epithelial	ATCC Cat#: CRL-2527		Yes
XLT-15		(Yaoita and Nakajima, 1997)	1997	CVCL_6744	<i>Xenopus laevis</i>	Unspecified	Tadpole	Tail tip	Spontaneous	Myoblast			No
XR1		(Sakaguchi et al., 1989)	1989	CVCL_T691	<i>Xenopus laevis</i>	Unspecified	Embryo	Retinal neuroepithelium	Spontaneous	Epithelial			No
XR6		(Sakaguchi et al., 1989)	1989	None	<i>Xenopus laevis</i>	Unspecified	Embryo	Retinal neuroepithelium	Spontaneous	Epithelial			No
XR8		(Sakaguchi et al., 1989)	1989	CVCL_T692	<i>Xenopus laevis</i>	Unspecified	Embryo	Retinal neuroepithelium	Spontaneous	Epithelial			No
XR9		(Sakaguchi et al., 1989)	1989	CVCL_T693	<i>Xenopus laevis</i>	Unspecified	Embryo	Retinal neuroepithelium	Spontaneous	Epithelial			No
XRpe1		(Sakaguchi and Henderson, 1993)	1993	CVCL_T694	<i>Xenopus laevis</i>	Unspecified	Embryo	Retinal pigment epithelium	Spontaneous	Epithelial			No
XRpe2		(Sakaguchi and Henderson, 1993)	1993	CVCL_T695	<i>Xenopus laevis</i>	Unspecified	Embryo	Retinal pigment epithelium	Spontaneous	Epithelial			No
XRpe4		(Sakaguchi and Henderson, 1993)	1993	CVCL_T696	<i>Xenopus laevis</i>	Unspecified	Embryo	Retinal pigment epithelium	Spontaneous	Epithelial			No
XRpe5		(Sakaguchi and Henderson, 1993))	1993	CVCL_T697	<i>Xenopus laevis</i>	Unspecified	Embryo	Retinal pigment epithelium	Spontaneous	Epithelial			No
XTC-2 [BirAV5]	XTC-2	None	2021	CVCL_C0NH	<i>Xenopus laevis</i>	Unspecified	Tadpole	Whole	Engineered to express the biotin ligase BirAV5 and <i>Xenopus</i> Krüppel-like factor 9 BIOfusion protein	Fibroblast-like		Kerafast Cat#: EMU040	No
XTC-2 [BirAV5-FLBIO-xKLF9]	XTC-2	None	2021	CVCL_C0NI	<i>Xenopus laevis</i>	Unspecified	Tadpole	Whole	Engineered to express the biotin ligase BirAV5	Fibroblast-like		Kerafast Cat#: EMU041	No
XTC-2		(Pudney et al., 1973)	1973	CVCL_5610	<i>Xenopus laevis</i>	Unspecified	Tadpole	Whole	Spontaneous	Fibroblast-like	RCB Cat#: RCB0771		Yes
XTC-GTX-11	XTC-2	(van den Eijnden-Van Raaij et al., 1990)	1990	CVCL_VQ56	<i>Xenopus laevis</i>	Unspecified	Tadpole	Whole	Spontaneous	Fibroblast-like			No
XTH-2		(Schlage et al. 1981)	1981	CVCL_1892	<i>Xenopus laevis</i>	Unspecified	Tadpole	Heart	Spontaneous	Endothelial	DSMZ Cat#: ACC-190		No
XTY		(Fukui et al., 1992)	1992	CVCL_5611	<i>Xenopus laevis</i>	Female	Adult	Subcutaneous tumour	Spontaneous	Fibroblast-like	RCB Cat #: RCB0770		No
LM6		(Plytycz et al., 1988)	1988	None	<i>Xenopus laevis</i> x <i>Xenopus muelleri</i>	Unspecified	Tadpole	Whole (without tail, intestines)	Spontaneous	Fibroblastoid			No
91.1.1.F1		(Sinzelle et al., 2012)	2012	CVCL_6559	<i>Xenopus tropicalis</i>	Unspecified	Tadpole	Hindlimb	Spontaneous	Epithelioid			No
XTN-10		(Gorbsky et al., 2022)	2022	CVCL_C0N6	<i>Xenopus tropicalis</i>	Unspecified	Embryo	Whole	Spontaneous	Unknown			Yes
XTN-12		(Gorbsky et al., 2022)	2022	CVCL_C0N7	<i>Xenopus tropicalis</i>	Unspecified	Embryo	Whole	Spontaneous	Unknown			Yes

XTN-6		(Gorbsky et al., 2022)	2022	CVCL_C0N4	<i>Xenopus tropicalis</i>	Unspecified	Embryo	Whole	Spontaneous	Unknown			Yes
XTN-8		(Gorbsky et al., 2022)	2022	CVCL_C0N5	<i>Xenopus tropicalis</i>	Unspecified	Embryo	Whole	Spontaneous	Unknown			Yes
Speedy	91.1.F1	(Sinzelle et al., 2012)	2012	CVCL_6730	<i>Xenopus tropicalis</i>	Unspecified	Tadpole	Limb bud	Spontaneous	Fibroblast			No