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The core promoter controls basal and inducible expression of duck retinoic acid inducible gene-I (RIG-I)

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ABSTRACT

Retinoic acid inducible gene-I (RIG-I) is a cytoplasmic RNA sensor for detecting a variety of RNA viruses including influenza A viruses. Detection ultimately produces Type I interferon (IFN), which stimulates expression of interferon stimulated genes (ISGs), including RIG-I itself in a positive feedback loop. The structure and function of RIG-I is conserved across phylogeny, despite significant protein sequence divergence, however, the promoter sequences do not show the expected phylogenetic relationships and it is not known whether they are similarly regulated. We previously cloned duck RIG-I and showed it is highly induced during influenza A infection consistent with induction by the interferon produced. Here, we identified the Pekin duck RIG-I promoter and constructed promoter reporter vectors, which we transfected into duck embryonic fibroblasts or chicken DF-1 cells and tested in dual luciferase assays. We showed that activation of the Mitochondrial Antiviral Signalling (MAVS) pathway using the constitutively active N-terminal region of RIG-I or polyinosinic-polycytidylic acid (poly I:C) led to stimulation of duck RIG-I promoter activity. Using deletion constructs we showed the core promoter lies in the proximal 250 basepairs, and we identified essential *cis*-regulatory elements, a GC-box and an interferon-sensitive response element (ISRE), responsible for basal and inducible expression, respectively. Using mCherry-tagged interferon regulatory factors (IRFs) cloned from chickens and ducks, we show overexpression of chIRF7 induced the duck RIG-I promoter, and this required the ISRE site. Finally, we also demonstrated that overexpressed chIRF7 translocated to the nucleus, which was augmented by MAVS activation using RIG-I 2CARD. Our findings demonstrate that RIG-I expression is induced by chIRF7, in a positive regulatory loop. These studies show that the duck RIG-I promoter is appropriately regulated in chicken cells, necessary for the potential generation of transgenic chickens expressing RIG-I.

1. Introduction

RIG-I-like receptors (RLRs) are broadly expressed in a variety of cell types in most tissues and are regarded as essential pattern recognition receptors for host recognition of various families of RNA viruses, such as Paramyxoviridae, Rhabdoviridae, Orthomyxoviridae, Filoviridae, and Coronaviridae (Loo and Gale, 2011). RLRs consist of three related cytoplasmic DExD/H box RNA helicase proteins; retinoic acid inducible gene-I (RIG-I), melanoma differentiation gene-5 (MDA5) and laboratory of genetics and physiology-2 (LGP2). RIG-I was the first identified and also the best characterized (Yoneyama et al., 2004). RIG-I is a critical pattern recognition receptor involved in recognition of RNA viruses (Kato et al., 2005), however it has a myriad of other roles which were recently reviewed (Matsumiya and Stafforini, 2010), including cellular differentiation (Liu et al., 2000), bacterial TLR4-stimulated phagocytosis (Kong et al., 2009), apoptosis (Chattopadhyay et al., 2016; Chattopadhyay and Sen, 2017) and inflammation (Kawai and Akira, 2006).

RIG-I is composed of two N-terminal caspase activation and recruitment domains (2CARDs), a central DExD/H box helicase domain and a C-terminal regulatory domain (CTD). In the absence of an RNA trigger, the 2CARDs fold back to the helicase and C-terminal domains to form an auto-repressed state. Upon binding of its ligand (short dsRNA or 5′-triphosphate ssRNA) (Hornung et al., 2006; Lu et al., 2010; Luo et al., 2011; Yoneyama et al., 2005) to the helicase domain and CTD, RIG-I undergoes extensive rearrangement to expose the 2CARDs (Luo et al., 2011). Mammalian RIG-I is activated upon ubiquitination by TRIM25 (Gack et al., 2007), an E3 ubiquitin ligase, and its 2CARDs interact with the CARD domain of the adaptor protein, mitochondria antiviral-signaling protein (MAVS) (Seth et al., 2005). MAVS

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Table 1
Primer list.

Name	Location	sequence (5′ to 3′)	Application
RIG-I promoter F1	(−1140 ~ −1118)	CCAAGTAAAAATGCCTCTCTGCT	duck RIG-I promoter amplification
RIG-I promoter R1	(+338 ~ +315)	GCATCGCGTCCAGCATCCCTCGGA	duck RIG-I promoter amplification
RIG-I promoter F2	(−2024 ~ −2003)	AGCTGATGACCTGCAAAAAGTT	duck RIG-I promoter amplification
RIG-I promoter R2-1	(+63 ~ +43)	GTTGAGGCTCCGCTCGATGTA	duck RIG-I promoter amplification
RIG-I promoter R2-2	(−1 ~ −19)	GGCTGGGCTCTGCCGGCCG	duck RIG-I promoter amplification
SDM R		AAGGGCGAATTCCAGCACACTG	*Serial deletion construct
1kb F	(−1001 ~ −980)	CTTACTGAAAATGCAGGGTGGA	p1000 construction
500bp F	(−501 ~ −480)	GAGCGGCGGAGACAAAGTGCCA	p500 construction
250bp	(−250 ~ −229)	CTGGACCCCAGGCCCGTGTCTC	p250 construction
125bp F	(−125 ~ −104)	ATCTCCACACCCCGCGGGGGCC	p125 construction
73bp F	(−73 ~ −52)	GCTGCCTTTCTGTGCCGAGCCG	p73 construction
ΔRandom F	(−192~ −172)	TCACGAAGCTGCGAGCCGCTG	random deletion as control
ΔRandom R	(−230 ~ −250)	AGACACGGGCCTGGGGTCCAG	random deletion as control
ΔNHLH1 F	(−178 ~ −158)	GCCGCTGCCTATGCCCGCTGG	NHLH1 binding site deletion
ΔNHLH1 R	(−191 ~ −211)	GAGCCTCGGATCGCCACCAAC	NHLH1 binding site deletion
ΔGC-box F	(−140 ~ −120)	GCGGATGGGGCCGCGATCTCC	GC-box deletion
ΔGC-box R	(−152 ~ −172)	TGGCCGCCAGCGGGCATAGGC	GC-box deletion
ΔIRSE F	(−89 ~ −71)	TTCCCCGCCGGGCCTCGCT	IRSE deletion
ΔIRSE R	(−104 ~ −122)	GGCCCCCGCGGGGTGTGGA	IRSE deletion
mGC-box F1	(−157 ~ −133)	CGGCCAGAGGGCTTGCTGCGGATGG	GC-box point mutation 1
mGC-box R1	(−133 ~ −157)	CCATCCGCAGCAAGCCCTCTGGCCG	GC-box point mutation 1
mGC-box F2	(−161 ~ −134)	CTGGCGGCCAGAGTTCTTGCTGCGGATG	GC-box point mutation 2
mGC-box R2	(−134 ~ −161)	CATCCGCAGCAAGAACTCTGGCCGCCAG	GC-box point mutation 2
mIRSE F1	(−113 ~ −87)	CGCGGGGGCCGCTAACGTTTTCCATTC	IRSE point mutation 1
mIRSE R1	(−87 ~ −113)	GAATGGAAAACGTTAGCGGCCCCCGCG	IRSE point mutation 1
mIRSE F2	(−107 ~ −80)	GGCCGCTAACGTGAGCCATTCCCCGCCG	IRSE point mutation 2
mIRSE R2	(−80 ~ −107)	CGGCGGGGAATGGCTCACGTTAGCGGCC	IRSE point mutation 2
M13F		CAGGAAACAGCTATGAC	pCR2.1-TOPO vector sequence
M13R		GTAAAACGA CGGCCAGT	pCR2.1-TOPO vector sequence
RVprimer 3		CTAGCAAAATAGGCTGTCCC	pGL3-Basic vector sequence
GLprimer2		CTTTATCTTTTTGGCGTCTTCCA	pGL3-Basic vector sequence
RIG-I promoter F	(−1329 ~ −1308)	CTCTTCTCAGGTGAAGGACAAG	RIG-I promoter internal sequence
RIG-I promoter R	(−729 ~ −750)	TTCTCTCACATTTTCTCACTCT	RIG-I promoter internal sequence
EcoRI-chIRF1 F		GAATTCAATGCCCGTCTCAAG	*chIRF-1 amplification
chIRF-1-KpnI R1		GGTACCTTACAAGCTGCAGGA	*chIRF-1 amplification
EcoRI-dIRF1 F		GAATTCAATGCCCGTCTCCAG	*dIRF-1 amplification
dIRF1-KpnI R		GGTACCTTACAAGCCACAGGA	*dIRF-1 amplification
HindIII-chIRF1 F		CCCAAGCTTCGAATTCAATGCCCGTCT	*chIRF-1 amplification 2
chIRF1-KpnI R2		CGGGGTACCTTACAAGCTGCAGGA	*chIRF-1 amplification 2
pmCherry F		AACATCAAGTTGGACATCACC	mCherry vector sequence
pmCherry R		GAAATTTGTGATGCTATTGC	mCherry vector sequence

F: forward primer; R: reverse primer; SDM: serial deletion mutation; Δ: deletion mutation; m: point mutation; d: duck; ch: chicken.

oligomerization stimulates a downstream signaling pathway leading to inflammatory cytokine and Type I IFN release, further inducing the production of interferon stimulated genes (ISGs), many of which are antiviral factors (Kawai and Akira, 2006). We have shown that duck RIG-I is also activated by TRIM25, albeit without necessarily attaching ubiquitin chains (Miranzo-Navarro and Magor, 2014). In ducks, as in humans, RIG-I CARD domains engage MAVS and oligomerization is necessary for signalling (Wu et al., 2014). Overexpression of the constitutively active N-terminal region of RIG-I, or MAVS alone can initiate signalling (Wu et al., 2014).

While RIG-I was initially identified as a novel retinoic acid inducible gene (Liu et al., 2000), it has been shown to be inducible by LPS (Imaizumi et al., 2002), IFN-gamma (Cui et al., 2004) and Type I interferon (Matikainen et al., 2006) and poly (I:C) (Kubota et al., 2006). Su and colleagues carried out a deletion analysis of the human RIG-I promoter and showed that a canonical IRF1 binding site located between nucleotides -17 and -4 was critical for interferon induction, and that IRF1 binds to this site by electromobility shift assay (Su et al., 2007). Recently, using STAT1-null mouse cells U3A, or Type I IFN receptor (IFNAR)-null cells U5A, it was shown that the early induction of RIG-I in response to a viral mimic, poly (I:C), involves IRF3 (Hayakari et al., 2016).

We previously cloned duck RIG-I and showed that it is critically involved in innate immunity of ducks to influenza virus (Barber et al., 2010). We showed that overexpressed duck RIG-I can functionally compensate for the lack of RIG-I in chicken cells, and turn on interferon and interferon stimulated genes. Further, duck RIG-I expressed in chicken cells can augment the expression of interferon-stimulated genes and reduce influenza replication (Barber et al., 2013). Duck RIG-I itself is highly induced by infection with highly pathogenic avian influenza, presumably through the action of interferons produced during infection in a positive feedback loop. The N-terminal end of duck RIG-I (2CARD) is constitutively active and can induce expression of an interferon reporter construct and downstream interferon-stimulated genes (Miranzo-Navarro and Magor, 2014). Together our work suggests that it might be worthwhile to make chickens transgenic for duck RIG-I to improve their ability to detect and respond to influenza infection. However, the expression of RIG-I must be strictly controlled, as inappropriate production of inflammatory cytokines will lead to inflammation-related autoimmune diseases or failure in viral clearance. To serve as a pattern recognition receptor RIG-I must be expressed in cells at a basal level, and be highly induced during infection. Ducks and chickens diverged early in avian evolution (Hackett et al., 2008), thus it is not known whether the duck RIG-I promoter will function in chicken cells, and whether appropriate basal and inducible expression can be achieved. Here we characterize the promoter of duck RIG-I for expression in chicken cells. We identify the transcription factor binding sites in the core promoter that control basal expression, and show chicken IRF7 stimulates inducible expression downstream of RIG-I signalling.