A transgenic mouse model for the simultaneous monitoring of ANF and BNP gene activity during heart development and disease

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Aim
The expression of Nppa (ANF) and Nppb (BNP) marks the chamber myocardium in the embryo, and both genes serve as early and accurate markers for hypertrophy and heart failure. Non-invasive visualization of Nppa–Nppb expression in living mice would enable to evaluate the disease state during the course of time in heart disease models. We sought to develop a method to assess the pattern and level of Nppa and Nppb expression within living mice.

Methods and results
A modified bacterial artificial chromosome containing a genomic segment spanning the Nppa–Nppb locus was randomly integrated into the mouse genome. Firefly Luciferase was inserted into Nppa and the red fluorescent protein gene Katushka into Nppb. Both reporters precisely recapitulated the spatio-temporal patterns of Nppa and Nppb, respectively. In a hypertrophy model (transverse aortic constriction) and myocardial infarction model (left anterior descending coronary artery occlusion), the non-invasively measured bioluminescent signal from Luciferase correlated with Nppa expression, and the intensity of red fluorescence with levels of the expression of Katushka and Nppb. After myocardial infarction, the border zone of the infarct area was readily identified by an increased intensity of Katushka fluorescence.

Conclusions
A genomic region sufficient to regulate the developmental pattern and stress response of Nppa and Nppb has been defined. The double reporter mice can be used for the functional imaging and investigation of cardiac hypertrophy and myocardial infarction in vivo.

Keywords
Natriuretic peptide • Hypertrophy • Myocardial infarction • Bioluminescent imaging • Double reporter

1. Introduction
Congestive heart failure (HF) is a common disabling condition that can lead to death. HF occurs when the heart is unable to adapt to haemodynamic changes and distribute blood flow to meet the needs of the body, and is the end stage of a variety of cardiac diseases. Atrial natriuretic factor (ANF) and brain natriuretic peptide (BNP) are diuretic and natriuretic hormones produced by the foetal and adult heart.1,2 Because ANF and BNP release from cardiomyocytes is induced by myocardial stress, the plasma levels of these peptides are used as diagnostic markers in the clinical setting to monitor the severity of hypertrophy and HF.3 At the same time, cardiac stress induces a strong ventricular expression of the genes encoding the natriuretic peptides (NPs), natriuretic peptide A (Nppa) and B (Nppb). Therefore, Nppa and Nppb are widely used markers for the hypertrophic response in cell culture4 or for the development of hypertrophy and HF-related diseases in animal models.5 Nppa and Nppb are expressed in the embryonic chamber myocardium and serve as markers for chamber differentiation.6 Ventricular Nppa expression strongly decreases after birth, whereas Nppb remains expressed in the adult ventricular myocardium. Increased cardiac load induces both Nppa and Nppb expression in the ventricles, which is widely referred to as the ‘reactivation of the foetal gene program’.7 Rat and human proximal promoter fragments of Nppb were shown to respond to angiotensin II stimulation and myocardial infarction (MI), respectively.8,9 However, the sequences controlling its full-developmental pattern and expression in the adult heart have not been identified. Proximal regions of the rat and mouse Nppa gene were not able to recapitulate either the foetal ventricular expression or the stress response.10,11

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2. Methods

An expanded methods section is available in Supplementary material online.

2.1 BAC modification

The BAC336 clone of a mouse 129 SvJ BAC library (Incyte, St Louis, MO, USA) harbouring the sequence from −141 to +58 kb relative to the transcription start site of Nppa was modified with Luciferase and Katushka reporter genes (Figure 1A). The sequences cccacgcgcAGggc and cggcATGgatc at the translation start sites of Nppa and Npbb were replaced by Luciferase and Katushka, respectively, using the BAC modification protocol described by Gong et al. Modified BAC was purified using a CsCl gradient and used for pronuclear injection.

2.2 Animals

BAC336LK mice (FVB) were generated by pronuclear injections of modified BAC. Animal care and experiments conform to the Directive 2010/63/EU of the European Parliament. All animal work was approved by the Animal Experimental Committee of the Academic Medical Center, Amsterdam, and carried out in compliance with the Dutch government guidelines.

2.3 In situ hybridization

Non-radioactive in situ hybridization (ISH) on 10 μm sections was performed as described previously using mRNA probes for the detection of Nppa, Npbb, Luciferase, Katushka, and cTnl.

2.4 RNA isolation and quantitative real-time PCR (qPCR)

Total RNA was isolated from atria and ventricular tissue of embryonic, adult control, transverse aortic constriction (TAC)-operated, and MI mice using Trizol Reagent according to the manufacturer’s protocol (Invitrogen). cDNA was generated using the Superscript II system (Invitrogen) and expression levels of different genes were assessed with qPCR using the LightCycler Real-Time PCR system (Roche Diagnostics, Almere, The Netherlands) and the primers described in Supplementary material online.

2.5 Transverse aortic constriction

TAC was performed in 8 to 12-week-old BAC336LK mice. Mice were anaesthetized with isoflurane (induction 4%, maintenance 2.5%). The aorta was subjected to a defined 27-gauge constriction as described before. Buprenorphone (50 μg/kg) was injected subcutaneously (s.c.) for post-surgical analgesia. Age-matched unbanded animals were used as controls. After 2 weeks, in vivo bioluminescence imaging was performed; mice were anaesthetized in a CO2/O2 mixture and subsequently killed by cervical dislocation. Body weight, heart weight (HW), and tibia length (TL) were determined and ventricular tissue was collected for RNA isolation, ISH, and luciferase activity assay.

2.6 Myocardial infarction

Infarction was created in 8 to 12-week-old BAC336LK mice. Mice were sedated with s.c. injected mixture of ketamine (60 mg/kg) and dexmedetomidine hydrochloride (0.35 mg/kg) and anaesthetized with 2% isoflurane. Atropin (0.5 mg/kg) was injected intraperitoneally (i.p.). Buprenorphine (68 μg/kg) was s.c. injected for analgesia. Infarction was created by permanent ligation of the left anterior descending coronary artery (LAD). After operation, atipamezole hydrochloride (2.5 mg/kg) was s.c. injected to antagonize dexmedetomidine hydrochloride. After 1 and 2 weeks, MI and non-operated mice, used as controls, were subjected to bioluminescence imaging and subsequently killed by cervical dislocation. Samples from the right ventricle, the interventricular septum, the left ventricle (control hearts), or corresponding border zone region (MI hearts) were collected 2 weeks after MI for RNA isolation.

2.7 In vivo bioluminescence imaging

In vivo bioluminescence imaging was performed on the Photon Imager (BioSpace Lab). Two weeks after TAC as well as 1 and 2 weeks after LAD occlusion, experimental and control mice were sedated with 4% isoflurane, i.p. injected with 100 μL saline solution of D-Luciferin (30 mg/mL) and anaesthetized with 2% isoflurane. Pseudo-colour bioluminescence images were acquired 10 min after D-Luciferin injection during the 10 min period. Images were analysed with the M3Vision software (BioSpace Lab).

2.8 Luciferase activity assay

Luciferase extract was prepared from the ventricular samples of control and TAC mice after 2 weeks. Relative light units were normalized to total amount of protein.

3. Results

3.1 Generation of a luciferase-red fluorescent protein double reporter for Nppa and Npbb activity

BAC336 spans the region from −141 to +58 kb relative to Nppa and was shown to contain stress/hypertrophy enhancers for Npbb. To identify distal regulatory sequences responsible for the developmental expression and reactivation of both Nppa and Npbb, we modified BAC336 with two reporter genes. Synthetic firefly Luciferase and dimeric bright red fluorescent protein Katushka were inserted into the Npbb translation start sites, respectively (Figure 1A). Insertion of the reporter genes and BAC integrity were assessed by restriction analysis of the modified regions (Supplementary material online, Figure S1A). Two mouse lines were generated with randomly integrated multiple copies of the Luciferase-Katushka BAC construct (BAC336LK). Integrity of BAC336LK within the mouse genome was confirmed by Southern blotting with two probes in the promoter regions of Nppa.
Figure 1  Overview of Nppa – Nppb locus and expression analysis of the double-reporter construct. (A) Overview of the gene positions with Polymerase II ChIP-seq data and genomic segment in BAC336. Modification of Nppb and Nppa within BAC336 with Katushka (Kat) and Luciferase (Luc) is shown in red and green, respectively. (B) Katushka and Luciferase mRNA expression mimics expression of Nppb and Nppa, respectively, in both atrial and trabecular ventricular myocardium in E15.5 embryonic hearts, whereas the sinus node, atrioventricular canal and outflow tract myocardium are correctly negative. Down-regulation of Nppa in the adult ventricles is recapitulated by Luciferase, whereas Nppb and Katushka remain to be expressed in the adult ventricles. (C and D) qPCR analysis of ventricular gene expression in two founder lines. Foetal and adult ventricular levels of Nppb and Katushka mRNA remained unchanged before and after birth, comprising 40 – 70% of atrial expression (C). Ventricular levels of Nppa and Luciferase mRNA significantly (P < 0.02; n = 3 for each founder line) decreased to 1% of atrial activity after birth (D). Error bars indicate SEM; ra, right atrium; la, left atrium; rv, right ventricle; lv, left ventricle; sn, sinus node; scale bar = 0.5 mm.
and Nppb, respectively (see Supplementary material online, Figure S1B and S1C). Mice heterozygous for Luciferase and Katushka are phenotypically normal and display the heart-specific expression of the transgenes (data not shown).

### 3.2 Luciferase and Katushka recapitulate the spatio-temporal expression patterns of Nppa and Nppb

BAC336 was shown to contain distal regulatory elements responsible for developmental expression and reactivation of Nppa, but Nppb developmental regulatory elements have not been established. To determine whether the expression patterns of Luciferase and Katushka recapitulate the expression of Nppa and Nppb, respectively, we performed ISH on sections of two independent BAC336LK lines. As shown in Figure 1B and Supplementary material online, Figure S2, both reporter genes precisely mimic the expression pattern of Nppa and Nppb in the foetal atria and the trabeculated layer of the ventricular myocardium. The expression of both the reporters and the endogenous genes was absent from the sinus node, atrioventricular canal, compact ventricular wall and outflow tract. Down-regulation of Nppa in the ventricles after birth to 1% of the atrial expression is recapitulated by down-regulation of Luciferase expression (Figure 1B and D; Supplementary material online, Figure S2), whereas Nppb and Katushka remain expressed in the adult ventricles at the same level as in the foetal heart (Figure 1B and C; Supplementary material online, Figure S2). We conclude that BAC336 contains all information required for the remarkably similar spatio-temporal expression patterns of Nppa and Nppb, and that the regulatory sequences driving developmental and tissue-specific expression of these genes are positioned within the same DNA region. We hypothesize that the reporter genes will allow monitoring the induction of Nppa and Nppb during disease in vivo.

### 3.3 In vivo bioluminescence is a sensor of Nppa induction in the ventricles during hypertrophy

Next, we tested whether Luciferase expression in the ventricles during hypertrophy mimics that of Nppa. In addition, we explored the possibility to measure light emission from Luciferase activity in living mice. Transgenic mice were subjected to TAC. Two weeks after TAC, the HW to TL ratio (HW/TL) and the level of hypertrophy marker genes Nppa and Nppb were increased in the TAC mice compared with the controls (Figure 2A and B; Supplementary material online, Figure S3A and B). Detection of Luciferase protein expression by in vivo bioluminescence imaging showed that the bioluminescent signal was restricted to the heart area and significantly increased in the TAC animals (Figure 2C and D; Supplementary material online, Figure S3C and D). An increased mRNA level of Luciferase correlated with the increased level of Nppa in the ventricles (Figure 2E and F; Supplementary material online, Figure S3E). Furthermore, activity of Luciferase protein measured in the ventricular samples of TAC animals increased compared with the control mice in agreement with the bioluminescent signal obtained from the whole hearts in vivo (Figure 2G; Supplementary material online, Figure S3F). Importantly, mRNA levels of Nppa significantly correlated with the bioluminescence response (T1 and T2 in Figure 2H; Supplementary material online, Figure S3G). The severity of hypertrophy 2 weeks after TAC as determined by functional parameters and the expression of hypertrophy markers varied between animals. Elevated Nppa expression correlated with increased left ventricular posterior wall thickness, and decreased fractional shortening and ejection fraction (see Supplementary material online, Figure S4). The increase in the HW/TL ratio and the levels of the expression of extracellular matrix components Collagen 1a1 and Collagen 3a1 (markers of fibrosis) correlated with the levels of the expression of Luciferase (see Supplementary material online, Figure S5) and the bioluminescent signal (Figure 2I–K). Taken together, Luciferase expression and protein activity during hypertrophy mimic reactivation of Nppa in the ventricles, and the bioluminescent signal measured non-invasively in vivo accurately senses induction of Nppa expression and the severity of hypertrophy.

### 3.4 Katushka fluorescence reflects Nppb up-regulation in the ventricles during hypertrophy

Nppb is up-regulated in the ventricular myocardium in animal models of pressure overload.22,23 (Figure 2B; Supplementary material online, Figures S3B and S4). To determine whether Katushka recapitulates Nppb up-regulation in the heart, we measured mRNA levels of these genes in the ventricular samples after 2 weeks of TAC. Katushka mRNA levels correlated with the severity of hypertrophy determined by the HW/TL ratio and fibrosis development (see Supplementary material online, Figure S5) and mimicked Nppb up-regulation in the ventricles during hypertrophy (Figure 2E). Katushka mRNA levels significantly correlated with those of Nppb (Figure 2L; Supplementary material online, Figure S3H) and increased production of Katushka protein could be readily visualized by the fluorescence signal in isolated hearts (Figure 2M; Supplementary material online, Figure S3I). Therefore, BAC336 contains cis-regulatory elements for correct up-regulation of Katushka in the stressed ventricular myocardium after TAC.

### 3.5 BAC336LK is a sensor of Nppa and Nppb during MI

Both Nppa and Nppb are up-regulated in the border zone after MI that leads to HF. Using the BAC336LK line, we examined the in vivo detection imaging potential of Luciferase and ex vivo properties of Katushka after MI. Similar to the hypertrophy model, the local up-regulation of Luciferase could be detected with bioluminescence imaging 1 week (data not shown) and 2 weeks after MI (Figure 3A). In turn, local induction of Katushka was identified right away by bright red fluorescence around the necrotic tissue (Figure 3B, MI1 compared with control). The variable response of mice to MI, dependent up on the location of LAD occlusion, was reflected in the bioluminescence and fluorescence imaging (Figure 3A and B, MI2 compared with MI1 and control). ISH staining of Luciferase and Katushka 1 week after operation confirmed that up-regulation of the reporter genes recapitulated local up-regulation of Nppa and Nppb in the border zone, respectively (Figure 3C). The necrotic infarct zone was marked by the absence of myocardium revealed by Ctril (Figure 3C). Ventricular mRNA levels of Luciferase and Katushka measured in the border zone, the interventricular septum and the right ventricle of control and infarcted hearts significantly correlated with the level of Nppa and Nppb in the corresponding tissue samples, respectively (Figure 3D and E). Moreover, mRNA levels of Nppa in the border zone of MI significantly correlated with the bioluminescent signal (Figure 3F). Hence, Luciferase and Katushka mimic Nppa and Nppb up-regulation, respectively, not only in hypertrophy, but also in MI models of HF. Nppb is up-regulated in the ventricles during hypertrophy progression and MI, preserves cardiac function, and serves as a marker of extensive fibrosis.25 Katushka expression correlated with the Collagen 1a1 and
Figure 2  Luciferase and Katushka sense ventricular expression of Nppa and Nppb, respectively, during hypertrophy in BAC336LK mice. (A) Increase of the HW/TL ratio in TAC mice (n = 4) compared with the controls (n = 4) 2 weeks after TAC surgery. *P < 0.05. (B) Up-regulation of Nppa (P < 0.01) and Nppb (P < 0.01) mRNA levels in the ventricles 2 weeks after TAC surgery was analysed with qPCR (n = 5 control and n = 7 TAC). (C) In vivo bioluminescence imaging of a representative control and two TAC mice 2 weeks after surgery. Increased luciferase activity was observed in the heart area of TAC mice (n = 6) compared with control mice (n = 4). (D) Quantification of light emission from BAC336LK control (n = 4) and TAC (n = 6) mice 2 weeks after surgery shows significant (P < 0.02) increase in bioluminescence in TAC mice. (E) Up-regulation of Nppa and Nppb in the left ventricular myocardium 2 weeks after TAC is mimicked by up-regulation of Luciferase and Katushka, respectively, as shown by ISH on sections. (F–L) Correlation of different measurements in ventricular samples of control and TAC hearts 2 weeks after surgery. Luciferase and Katushka mRNA levels significantly correlated with those of Nppa (F) and Nppb (L), respectively. Bioluminescent signal measured in vivo significantly correlated with the luciferase activity (G), Nppa mRNA levels (H), heart size (I) Collagen 1a1 (J), and Collagen 3a1 (K) mRNA levels in the ventricles. Highlighted samples from the control (C1), TAC1 (T1), and TAC2 (T2) mice correspond to the Figure 2C. (M) Red fluorescence of Katushka protein increased in the left ventricular myocardium of the TAC hearts compared with the controls. Two representative hearts are shown. Error bars indicate SEM; lv, left ventricle; scale bar = 0.5 mm (E) and 2 mm (M).
Figure 3  BAC336LK is a sensor of Nppa and Nppb during MI. (A) In vivo bioluminescence imaging of a representative control and two MI mice 2 weeks after LAD occlusion. Increased luciferase activity was observed in the heart area of MI mice (n = 4) compared with control mice (n = 5). (B) Red fluorescence of Katushka protein points out the localization of the infarcted area and the border zone 2 weeks after MI. LAD occlusion is shown with an arrow. (C) Up-regulation of Nppa and Nppb in the border zone after MI is mimicked by up-regulation of Luciferase and Katushka, respectively, as shown by ISH on sections. A representative control heart and a heart after 1 week MI are shown. In the right column, cTnl reveals the myocardium. (D–F) Correlation of different measurements in the ventricular samples of control (n = 5) and MI (n = 8) hearts 2 weeks after surgery. Luciferase and Katushka mRNA levels significantly correlated with those of Nppa (D) and Nppb (E), respectively, in the right ventricular, left ventricular and interventricular septum samples. Bioluminescent signal measured in vivo significantly correlated with Nppa levels measured by qPCR in the left ventricle of control and the border zone of MI mice. Highlighted samples from the control (C), MI1 and MI2 mice correspond to Figure 3A and B. RA, right atrium; LA, left atrium; RV, right ventricle; LV, left ventricle; IVS, interventricular septum; BZ, border zone; IZ, infarct zone; scale bar = 0.2 mm (C, upper panel), 0.05 mm (C, middle and lower panel), and 2 mm (B).
Collagen 3a1 expression in the border zone of MI surrounding the fibrotic scar (see Supplementary material online, Figure S6). In contrast, Luciferase expression did not show a significant correlation, although the bioluminescent signal was stronger in hearts with higher levels of the expression of the fibrosis markers (see Supplementary material online, Figure S6). Therefore, Luciferase can be used for direct in vivo, and Katushka for rapid ex vivo, monitoring of MI progression.

4. Discussion

In this study, we generated Nppa/Nppb double reporter transgenic mice by randomly integrating a BAC modified to contain Luciferase and Katushka. We showed that these mice are suitable to visualize the dynamic expression pattern of stress response marker Nppa by non-invasively monitoring the heart-specific bioluminescence of Luciferase in vivo. We also demonstrated the feasibility to visualize the infarct border zone on the basis of Katushka fluorescence. Moreover, double modification of the BAC revealed that the regulatory sequences for the developmental pattern and stress response of both Nppa and Nppb are localized within the same 198 kb DNA region.

4.1 A dual-colour reporter for the imaging of tissue-specific pathophysiological states

Great progress has been made in in vivo reporter analysis with the development of multicolour imaging. Different fluorescent proteins, as well as different luciferase enzymes, have been used to answer diverse biological questions in cancer research, gene and cell therapy, signal transduction, and gene regulation. Although fluorescence provides a wide spectrum of applications, bioluminescence imaging has the higher signal-to-background ratio, is highly sensitive, readily accessible, and allows reducing the number of experimental animals by avoiding their euthanization at several time points. The firefly luciferase (Photinus pyralis) is considered thus far to be the optimal choice in in vivo bioluminescence imaging compared with Gussia, Renilla, and bacterial luciferases. Dual imaging utilizing green emitting firefly luciferase with its red-shifted mutant requires appropriate filters and a special algorithm to separate the green emission overlap with the red filter, which is particularly important when two signals have a different intensity. Combination of firefly luciferase and far-red fluorescent protein Katushka for in vivo imaging in this study made it possible to avoid the issues described above. Disadvantages of bioluminescence imaging associated with the light absorption by melanin in skin and fur were avoided by using white-coated FVB mice in this study.

Far-red fluorescent protein Katushka demonstrates high brightness, fast maturation, high pH-stability, and photostability, which make it the protein of choice for whole-body imaging. However, we were not able to detect Katushka fluorescence from the heart tissue within a living animal. The fluorescence signal can be limited by the expression level of the protein. In addition, the appropriate combination of excitation laser wavelength, its position, and the detection system may affect sensitivity of the assay. Nevertheless, ex vivo fluorescence imaging of the BAC336LK mouse hearts revealed the possibility to detect up-regulation of Nppb during hypertrophy and precisely locate the border zone of MI without additional quantitative and histological techniques.

4.2 Monitoring of Nppa and Nppb expression during development, hypertrophy, and MI

This is the first study, to our knowledge, which demonstrates the use of a double reporter line to monitor both Nppa (in vivo) and Nppb (ex vivo) in murine models of HF-related diseases. Transgenic animals with promoter—reporter constructs based on the proximal promoter of Nppa (0.5 to 3.4 kb) only partially recapitulated foetal and adult endogenous Nppa expression, and failed to be reactivated in in vivo disease models. The use of a larger BAC-EGFP construct revealed that distal elements are required and sufficient to regulate Nppa expression during hypertrophy. Here, we introduced Luciferase and Katushka into the Nppa and Nppb translation start sites, respectively, in this BAC for simultaneous visualization of both genes in mice. Luciferase and Katushka correctly mimicked Nppa and Nppb in developing and the adult heart (Figure 1 and Supplementary material online, Figure S2), making them reliable to be used in monitoring developmental chamber differentiation and stress-induced up-regulation.

The development of HF is a complex systemic response of the organism to different stimuli, such as pressure or volume overload, hypoxia, and imbalance in neuro-humoral factors. Therefore, in vitro systems based on cardiomyocyte-derived cell lines (H9C2, HL-1), primary culture of rat neonatal cardiomyocytes, ESC-derived cardiomyocytes, or even engineered cardiac tissue might be challenging and not accurate for investigation of the cardiomyocyte response to stress. In vivo studies are important as an intermediate stage in translational research. Becker et al. developed an in vivo system in zebrafish to screen chemical modifiers of hypertrophic signalling based on the proximal 4 kb Nppb promoter, coupled to the luciferase reporter, which makes the zebrafish model attractive for cardiovascular science. In addition, identification of genetic variations causing cardiovascular defects and ease of genetic manipulation in zebrafish allow studying the mechanisms of human cardiac disorders. However, in contrast to mammals, adult zebrafish have a low pressure heart, a trabecular ventricle, and ventricular Nppa expression is maintained. Therefore, investigation of systemic response of the heart caused by hypertension, volume, and pressure overload is preferentially performed in mammals.

The use of promoter—reporter transgenic lines can be complicated by positioning effect, which has been largely overcome in our study by using a large 198 kb genomic segment to control Luciferase expression. In two independent founder lines, Luciferase executed the correct developmental program of Nppa expression and sensed its up-regulation in the ventricles during hypertrophy (Figure 2F and H). Even though the levels of the expression of Nppa and Luciferase in the ventricles are 1% of those in the atria, changes in the ventricular expression levels correlated with the bioluminescent signal recorded from the whole heart. We propose that this is due to greater up-regulation of the genes in the ventricles compared with the atria (data not shown), to the ventricular mass that is ~ 10 times larger than that of the atria and to the position of the ventricles much closer to the ventral body wall compared with the atria.

Although one of the lines had more variability in the levels of ventricular Luciferase expression (see Supplementary material online, Figure S3E), Luciferase still could serve as a marker of the disease progression (see Supplementary material online, Figure S3G). This model offers an opportunity to study any cardiovascular disorder involving up-regulation of Nppa in the heart. Crossing the BAC336LK line with other models of cardiovascular disorders will allow non-invasive monitoring of disease progression and response to pharmacological genetic interventions.
The MI model demonstrates that the local up-regulation of Nppa only in the border zone of MI can be visualized with bioluminescence imaging as well (Figure 3A). In the hypertrophy model, Nppa is massively up-regulated in all myocardium surrounding the left ventricular cavity. In contrast, in the MI model Nppa induction is highly localized within the ventricular wall, and the MI varies in severity, which may hinder quantification of the bioluminescent signal. However, stress-mediated up-regulation of Nppa in the left ventricle significantly correlated with the bioluminescence signal recorded from the whole heart in both hypertrophy and MI models (Figure 2H and 3F). This makes bioluminescence imaging an excellent tool to study the mechanisms of myocardial stress response in vivo.

Nppb is up-regulated earlier than Nppa in the acute phase of ventricular overload and is also a more sensitive marker of progression from stress response in vivo.

4.3 Nppb regulatory sequences for the developmental pattern and stress response are localized within the Nppa regulatory domain

Many Nppa promoter reporter constructs have been tested in vivo in search for stress-regulatory regions of the gene and mechanisms of HF. Less information is available about the in vivo regulatory elements of Nppb. Rat and human proximal promoter fragments of Nppb were shown to respond to angiotensin II stimulation and MI, respectively. However, the sequences controlling its full-length developmental pattern and expression in the adult heart have not been identified. Nppa and Nppb have arisen through tandem duplication of an ancestral NP gene, and, as a consequence, this locus may contain regulatory elements controlling the expression of both genes. Examples of genes arisen through duplications and regulated by shared elements are the Irx, Inx, and the Hox clusters, and the β-globin locus. The spatio-temporal and stress-induced expression patterns of Nppa and Nppb are strikingly similar (this study) and our study revealed that an 198 kb mouse genomic region contains all necessary information for these patterns of both genes. Together, these data suggest that Nppa and Nppb may be regulated by shared regulatory sequences within the 198 kb genomic fragment. Generation and analysis of transgenic mice with the modified BAC336LK in which deletions or truncations have been introduced will allow the identification of these elements. In summary, combination of two reporters, Luciferase and Katushka, in one mouse allows simultaneous monitoring of the expression of Nppa and Nppb, respectively. Bioluminescence imaging has become an important component of biomedical research and is now implemented in the studies of HF-related disorders.

Supplementary material

Supplementary material is available at Cardiovascular Research online.

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