Chapter 4

FLUORESCENCE RESONANCE ENERGY TRANSFER (FRET) STUDY ON PROTEIN-PROTEIN INTERACTION IN SINGLE LIVING CELLS

Xun Shen¹, Chunlei Zheng¹, Ziyang Lin², Yajun Yang¹ and Hanben Niu²

1. INTRODUCTION

The cell can be conceived as a biochemical information-processing device whose response to the environment depends on the state of spatially organized networks of protein activities. The interconnectivity and spatial organization of protein systems that sustain basic cellular functions is only maintained in the context of the whole intact molecular architecture of the cell. It is clear that understanding cell function by integrating molecular activities within the living cell is a big challenge for modern biology. Numerous biochemical assays revealing protein modifications, interactions or transport have already helped to bring us closer to this goal. However, none of the methods that are based on reconstituted systems in the test-tube can fully take into account the compartmentalized and interconnected nature of these reactions in cells. It is therefore necessary to develop methods that can measure the dynamics of these biochemical reactions in the intact cell and thereby extract the spatial organization in vivo. Only recently, and most probably because of the availability of genetically encoded fluorescent proteins, it has become possible to image not only cellular processes such as protein translocation or transport but also basic reactions such as protein interactions, proteolysis and phosphorylation in intact cells. In this chapter, we will introduce a newly developed technology, which is called fluorescence resonance energy transfer, for studying protein-protein interaction in single living cell through an example in which the interaction of the small heat shock protein 27 (hsp27) with p38, the member of the

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¹ Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China. shenxun@sin5.ibp.ac.cn
² Institute of Optoelectronics, Key Laboratory of Opto-electronics Devices and Systems, Shenzhen University, Ministry of Education, Shenzhen 518060, China.
mitogen-activated protein kinases (MAPK) family is concerned.

The current methods to investigate protein interaction mainly include yeast two-hybrid system (Luban and Goff, 1995) and co-immunoprecipitation (Anderson, 1998). The former employs a transcriptional ‘read-out’ in yeast and relies on the discovery that virtually any pair of proteins that interact with each other may be used to bring separate activation and DNA-binding domains together to reconstitute a transcriptional activator if one could be fused to a specific DNA-binding domain and the other to a transcriptional activation domain (Fields and Song, 1989). However, the requirement for stable expression of the fusion proteins and that the fusion proteins must be capable of transport to the nucleus seriously limits the application of this method. In co-immunoprecipitation study, the agarose beads coated with a selective antibody to precipitate a known protein from crude cell extract, then other specific antibodies are used to identify if any other unknown protein is co-precipitated with the known protein. Thus, if protein X forms a stable complex with protein Y in cell, immunoprecipitation of X may result in co-precipitation. However, the study of protein-protein interaction with this method can only be performed in vitro. Non-specific binding of two proteins cannot be excluded, and the low-affinity or transient binding of proteins is hardly to be detected. Besides those, co-precipitation can not applied to study the interaction involving insoluble protein.

The ‘green revolution’ initiated by the introduction of the green fluorescent protein GFP from Aequorea victoria (Chalfie et al., 1994) and the later developments of GFP-mutants possessing different spectral properties (Pollok and Heim, 1999) offered the possibility of simultaneous expression of donor and acceptor fusion proteins in the same cell and allowed measurement of their interactions by fluorescence resonance energy transfer (Miyawaki, 1997; Mitseli and Spector, 1997). At present, the combination of CFP (cyan fluorescence protein) donor and YFP (yellow fluorescence protein) acceptor fusion proteins are particularly useful. This effective FRET pair can be used to monitor the proximity of the two attached fluorescent proteins in 3-6 nm (Tsien, 1998). Co-expression of CFP- and YFP-fusion proteins has been successfully used to analyze short-time changes in protein-protein interactions, e.g. oligomerization, colocalization, complex formation (Ellenberg et al, 1999), activation of protein kinases (Ng et al. 1999) and mapping of enzyme activities in living cells (Bastiaens and Pepperkok, 2000).

2. PRINCIPLE OF FLUORESCENCE RESONANCE ENERGY TRANSFER (FRET)

FRET was first described by Förster (1948). It has become extremely important for modern cell biology, because FRET allows measuring distances between molecules on a scale of a few nanometers. This is far below the resolution limit of modern optical far field microscopy, which currently is at approximately 100 nm. Forster’s theory explains FRET as a dipole-dipole interaction between neighboring molecules and derives the dependence of the energy transfer efficiency $E$ on their actual proximity $R$. For efficient FRET the distance $R$ between these two molecules, the excited donor $D$ and the fluorescent acceptor $A$, is typically 2-7 nm. The direct non-linear dependence between $E$ and $R$ can be described as

$$E = \frac{1}{1+(R/R_0)^6} \quad (1)$$
where $R_0$ is the distance between the D and A for $E=0.5$. $R_0$ reflects the properties of a particular D-A pair including acceptor quantum yield, spectral overlap between D-emission and A-excitation and the relative spatial D-A orientation.

The extreme sensitivity of the FRET process on the distance between molecules 6th power dependence on $R$ renders it a very useful tool for the resolution of intracellular arrangement and dynamics of biological molecules. However, fluorophore bleaching and induction of cell damage limit the application of the FRET technique for the study of long-term (minutes to hours) processes of transport or signal transduction in living cells. The problem can be ameliorated by applying two-photon excitation instead of one-photon excitation. Since FRET occurs over distances similar to the size of proteins, it can be used to extend the resolution of the fluorescence microscope (typically 250 nm) to detect protein-protein interactions. FRET microscopy is thus an ideal technique to determine whether proteins that are co-localized at the level of light microscopy interact with one another in cells.

The CFP and YFP are most widely used donor and acceptor chromophores for construction of the fused protein(s) containing the investigated protein(s) in FRET study. Currently, there are two types FRET: intramolecular and intermolecular FRET. In intramolecular FRET, both the donor and acceptor chromophores are on the same host molecule, which undergoes a conformation transition or as substrate for an investigated enzyme. Any conformational change or cleavage of the molecule may yield a large change or even lose of the FRET. In intermolecular FRET, one investigated molecule (protein A) fused to the donor (CFP) and another molecule (protein B) fused to the acceptor (YFP). When the two proteins bind to each other, FRET occurs. When they dissociate, FRET diminishes. In FRET experiments, a single transfection (intramolecular FRET) or co-transfection (intermolecular FRET) of the constructs must first be performed. The occurrence of FRET can be observed by exciting the sample at the donor excitation wavelengths while measuring the fluorescence intensities emitted at wavelengths corresponding to the emission peaks of the donor versus those of the acceptor. If the acceptor and donor are at a favorable distance and orientation, donor emission intensity decreases (CFP, cyan) while the acceptor emission (YFP, yellow).

Figure 1. The principle of intermolecular FRET for studying protein-protein interaction. (cited from van Roessel, P and Brand)
intensity increases. Figure 1 depicts the fluorescence intensity changes of the donor and acceptor before and after FRET takes place.

3. FRET MICROSCOPY: METHODS FOR FRET MEASUREMENT

FRET results in several measurable phenomena, including sensitized acceptor fluorescence, quenching of donor fluorescence, and decrease in donor fluorescence lifetime. There are many ways to measure FRET in a microscope. These methods can be divided into intensity-based methods and lifetime-based methods.

3.1. Sensitized Acceptor Fluorescence

The fluorescence from the acceptor is measured while exciting the donor. This is typically done using three sets of filters which consist of CFP channel (excited at the excitation wavelength of CFP and measuring fluorescence at the emission wavelength of CFP), YFP channel (excited at the excitation wavelength of YFP and measuring fluorescence at the emission wavelength of YFP) and FRET channel (excited at the excitation wavelength of CFP and measuring fluorescence at the emission wavelength of YFP). However, the net FRET signal must be corrected against bleed-through of the non-FRET CFP fluorescence and the YFP fluorescence due to excitation at the excitation wavelength of CFP but not to the energy transfer from excited CFP according to the formula:

\[
\text{Net FRET signal} = \text{FRET signal} - a \cdot \text{YFP signal} - b \cdot \text{CFP signal}
\]

where \(a\) and \(b\) are the ratio of the signal in FRET channel to the signal in YFP channel in the absence of donor and to the signal in CFP channel in the absence of acceptor respectively. The coefficient \(a\) and \(b\) can be obtained from the measurement in the cells transfected with CFP fusion protein or YFP fusion protein alone. Obviously, a positive net FRET signal detected in the cells transfected with both fusion proteins indicates a fluorescence resonance energy transfer from donor to acceptor.

3.2. Acceptor Photobleaching Approach

FRET can also be accomplished by comparing the donor fluorescence intensity in the cells transfected with both CFP fusion protein (donor) and YFP fusion protein (acceptor) before and after destroying the acceptor by photobleaching. If FRET is initially present, fluorescent intensity of donor will increase after acceptor is photobleached. Energy transfer efficiency, \(E\), can be calculated according to following formula:

\[
E = 1 - \frac{F_{DA}}{F_D}
\]

with \(F_{DA}\) the fluorescence intensity of the donor in the presence of the acceptor and \(F_D\) the fluorescence intensity of the donor after acceptor is photobleached. An advantage of this method is that it requires only a single sample and that the energy transfer efficiency can thus be directly correlated with donor fluorescence before and after photobleaching of the acceptor.
3.3. Fluorescence Lifetime Imaging Microscopy (FLIM)

An alternative method is fluorescence lifetime imaging. Fluorescence lifetime of the donor is reduced by FRET and this effect can be directly measured by fluorescence lifetime imaging microscopy (FLIM) (Bastiaens and Squire, 1999). Fluorescence lifetime imaging microscopy is a technique in which the mean fluorescence lifetime of a chromophore is measured at each spatially resolvable element of a microscope image. The nanoseconds excited-state lifetime is independent of the chromophore concentration or light path length but dependent upon the excited-state reaction such as fluorescence resonance energy transfer. Imaging using fluorescence lifetimes may also provide functional data about the protein being probed since the lifetime of a fluorophore can be a function of its microenvironment within cell. Fluorescence lifetime imaging can be achieved by frequency domain techniques or by time-domain techniques. Frequency domain techniques use the phase shift between the modulated or pulsed excitation and the emission of the sample at the fundamental modulation frequency or its harmonics. Lifetime imaging can be achieved by modulated image intensifiers and wide-field microscopes (Squire et al., 2000) or by modulating single channel detectors used in laser scanning microscopes (Carlsson and Liljeborg, 1998). Time-domain techniques use pulsed excitation and record the fluorescence decay function directly. Lifetime imaging is achieved by gated image intensifiers (Cole et al., 2001), by directly gated CCDs (Mitchell et al., 2002), by counting the photons in several parallel time gates (Sytsma et al., 1998) or by time-correlated photon counting (Becker et al., 2004).

Instead of measuring emission intensity, the fluorescence lifetime of the donor alone ($\tau_D$) and also in the presence of the acceptor ($\tau_{DA}$) are measured. If FRET occurs, $\tau_{DA}$ will be different from $\tau_D$ and this difference can be used to calculate FRET efficiency $E$ as

$$E = \frac{1 - \tau_{DA}}{\tau_D}$$

The major advantage of FLIM is that it permits an internally calibrated measurement of FRET. Also, as only donor emission is monitored, factors that affect the quantum yield of the acceptor can be disregarded.

4. STUDIES ON THE INTERACTION BETWEEN HEAT SHOCK PROTEIN 27 AND P38 MAP KINASE

4.1. Small Heat Shock Protein 27 and p38 MAP Kinase

The 27 kDa stress response small heat shock protein 27, a marker of differentiation and proliferation, helps the cell in repair processes after environmental stress such as heat, UV-irradiation and oxidative stress. It has shown activation-dependent translocation from the cytosolic to the nuclear region and has been linked to the cellular stress response. It plays many other roles in the regulation of cell function, such as inhibiting death receptor-mediated apoptosis (Ricci et al., 2001), promoting growth of human astrocytomas (Khalid et al., 1995), characterizing the tumor as relatively benign and slow progressing (Bayerl et al., 1999). p38 (the $\alpha$-isoform) is a member of mitogen-activated protein kinase (MAPK) family and often activated by stress and various cytokines. It contains the phosphoacceptor sequence Thr-gly-tyr. In the signaling cascade, it is
activated via phosphorylation of Thr and Tyr residues by a dual-specificity serine-threonine MAPK-kinase (MKK), and then phosphorylates and activates MAPK-activated protein kinase-2 (MK2). The later then phosphorylates hsp27 (Freshney et al., 1994; Rouse et al., 1994). It was reported that when shock protein hsp27 became phosphorylated, the intracellular distribution of hsp27 was changed from the cytoplasm to the peri-nuclear region (Nakatsue et al., 1998). However, it has not been known if there is any direct interaction between hsp27 and p38. In this study, by using FRET technology we demonstrated that p38 could directly interact with hsp27 and the interaction depended on the activation of p38 and phosphatidylinositol 3-kinase (PI3K).

4.2. Experimental Procedures

4.2.1. Expression Vectors and Cell Transfection

Hsp27 cDNA and p38 cDNA were obtained by PCR amplification from vector, pBluescript-hsp27, and pCDNA3-Flag-p38 respectively. The CFP-hsp27 fusion construct was made by subcloning Hsp27 cDNA into cyan fluorescence protein (CFP) expressing vector, pECFP-C1, in EcoRI-BamHI sites. The p38 cDNA was subcloned into yellow fluorescence protein (YFP) expressing vector, pEYFP-C1, in HindIII-Xhol sites to construct the YFP-p38 fusion protein-expression vector. A glycine was inserted between YFP and p38 as linker in the YFP-p38 fluorescence chimera.

The mouse fibroblast cells (L929 cell line) were planted on glass-bottomed dishes (MatTek Corp.) and transfected with either CFP-hsp27 plasmid or YFP-p38 plasmid DNA or both using Lipofectamine 2000. 12 h after transfection, the full-length proteins (CFP-hsp27 or/and YFP-p38) expressed from each chimera can be observed in some cells.

4.2.2. Microscopy

As shown in Figure2, all FRET microscopic observations were performed on a Leica DM IRE2 confocal laser scanning microscope system at 37 °C 12 h after transfection. Excitation was provided by multimode argon ion laser beam using a double 458 nm/514 nm diachronic splitter. Donor (CFP) was excited at 458 nm and its fluorescence was detected in a bandwidth of 478-498 nm (CFP channel), whereas the excitation at 514 nm and emission at 545±15 nm were used for detecting acceptor (YFP) (YFP channel). For FRET, the excitation was at 458 nm and detection at 545±15 nm (FRET channel). The fluorescence images of the transfected cells were taken up at CFP-, YFP- and FRET-channel respectively. The FRET signal was corrected against bleed-through of the non-FRET CFP and YFP fluorescence.

Time-correlated single photon counting (TCSPC) fluorescence lifetime imaging using a multi-photon confocal laser scanning microscope system (Leica DM IRE2 and Becker & Hickl SPC730) was performed to obtain the donor CFP fluorescence lifetime images in the cells transfected with CFP-hsp27 chimera alone and co-transfected with both CFP-hsp27 and YFP-p38. The femto-seconds pulsed laser (Coherent Mire 900) beam was used to excite donor CFP in the transfected cells. The laser power was adjusted to give an average photon-counting rate of 10^4-10^5 photons·s⁻¹. Cells were imaged for 50 s to achieve appropriate photon statistics for determination of the fluorescence dynamics.
Figure 2. The fluorescence microscopy imaging system for FRET studies. It consists of a Leica TCS SP2 confocal laser scanning microscope, a time-correlated single photon counting unit (TCSPC), an argon ion laser, a femto-seconds pulsed laser (76 MHz, 120 fs) and two displayers for image intensity and lifetime measurement respectively.

4.3 Results

4.3.1. Interaction of p38 and Hsp27 in Quiescent Cell

As shown in Figure 3, the fluorescence from CFP and YFP can be observed simultaneously in the cell transfected with both CFP-hsp27 and YFP-p38 fusion chimeras. It can be seen that the heat shock proteins (hsp27) are distributed in cytoplasm, while the p38 MAP kinase in both cytoplasm and nuclei. The net fluorescence signal observed in FRET channel indicates the existence of an energy transfer from CFP to YFP.

Figure 3. Fluorescence images of the cell transfected with both CFP-hsp27 and YFP-p38 detected in CFP-, YFP- and FRET-channels respectively.
After bleaching CFP channel
Ex: 458nm
Em: 478-498nm

YFP channel
Ex: 514nm
Em: 530-560nm

FRET efficiency=1-F<sub>0</sub>/F<sub>0</sub>=0.32

Figure 4. The fluorescence intensity images of CFP-hsp27 and YFP-p38 in the L929 cell expressing the two fusion proteins before and after photobleaching of YFP.

In order to determine the FRET efficiency, dequenching of the donor CFP by selective photobleaching of the acceptor YFP was performed. In the experiments, the transfected cells were illuminated at the YFP excitation wavelength (514 nm) for 2 min at full laser power, and then CFP-hsp27 images were taken up at the same focal plane. To quantify changes in the CFP and YFP fluorescence intensity before and after bleaching, selected regions of the images were quantified using Leica confocal software. As shown in Figure 4, the fluorescence intensity of CFP significantly increased after photobleaching of YFP. Based on about 47% increase in the fluorescence intensity of CFP after photobleaching the acceptor YFP, the FRET efficiency of 32% was found between these two fusion proteins.

The FRET between these two fusion proteins was also studied by fluorescence lifetime imaging microscopy. The fluorescence intensity images and lifetime images of CFP in the cells transfected with CFP-hsp27 alone or with CFP-hsp27 and YFP-p38 together is shown in Figure 5. It is difficult to judge whether the fluorescence intensity of CFP in the cell expressing both the CFP-hsp27 and YFP-p38 fusion protein is less than that in the cell expressing the CFP-hsp27 fusion protein alone only by intensity measurements. However, the lifetime imaging clearly shows the shortening of the fluorescence lifetime of CFP when the CFP-hsp27 fusion protein is co-expressed with YFP-p38 fusion protein in cell. The FLIM measurement seems much better than fluorescence intensity measurement for showing the interaction between hsp27 and p38.

4.3.2. Interaction of p38 with Hsp27 in H<sub>2</sub>O<sub>2</sub>-stimulated Cell

The results in previous paragraph only show existence of the interaction of p38 with hsp27 when p38 MAP kinase is not activated. In order to know if the interaction
depends on the activity or phosphorylation of the MAP kinase, hydrogen peroxide was used to stimulate the cell co-transfected with the CFP-hsp27 fusion protein and YFP-p38 fusion protein. The H2O2-stimulation will result in an activation or phosphorylation of p38. Thus, the FRET study on the stimulated transfected cells may tell us more information on the regulation of the interaction. At first, the fluorescence intensity imaging approach was performed with the cell expressed both fusion proteins. 1 mM H2O2 was used to stimulate the cells, intensity images before stimulation and at various time after the stimulation were taken in CFP-channel and FRET-channel simultaneously. The results are shown in Figure 6. It can be seen that after stimulation the fluorescence intensity in CFP-channel became increasing while the intensity in FRET-channel was decreasing. The results suggested that the interaction between p38 and hsp27 becomes weakening upon activation of p38 MAP kinase.

Same as seen in the FLIM results in study of FRET between the two fusion proteins in quiescent cells, the FLIM approach is proved to be much better for studying the dynamic process of the interaction between the CFP-hsp27 and the YFP-p38 fusion proteins in the stimulated cells. Figure 7 shows the fluorescence intensity images and lifetime images of CFP in the cell expressing both fusion proteins at various moments after the stimulation by H2O2. It is very straightforward to see the dynamic change of the interaction as a gradual increase of the fluorescence lifetime of CFP after stimulation. It was observed that the average fluorescence lifetime of CFP increased from 1.17 ns before H2O2-stimulation to 1.34, 1.52 and 1.74 ns at 6, 12 and 18 min after the stimulation. The results suggest that the interaction of p38 with hsp27 is getting weak and weak as p38 MAP kinase being activated by H2O2. The results clearly demonstrate that activation or phosphorylation of p38 will lead to diminish of the interaction between p38 and hsp27.
To further testify the p38 activity-dependent interaction between p38 and hsp27, a selective inhibitor of p38, SB203580, was used to treat the cells before stimulation by H2O2. Figure 8 shows the observed fluorescence intensity images and lifetime images of CFP in the cell, which has two fusion proteins expressed and has been pre-incubated with 2 \( \mu \text{M} \) SB203580 for 20 min before stimulation, at various moments after the stimulation by H2O2. It was found that p38 inhibitor prevent the lifetime of CFP from increase when cell was stimulated by H2O2. This indicates that if p38 is unable to be activated, H2O2-stimulation would not affect the interaction between hsp27 and p38.
Figure 8. The fluorescence intensity images (up panels) and the fluorescent lifetime images (lower panels) of CFP in the L929 cell, which has both the CFP-hsp27 and YFP-p38 fusion proteins expressed and has been pre-incubated with 2μM SB203580 for 20 min before stimulation, after stimulation by 1 mM H₂O₂.

4.3.3. Interaction of p38 with hsp27 in the Cell Stimulated by Arachidonic Acid

Since the serine/threonine kinase protein kinase B, also called Akt, is found to exist in a signaling complex containing p38 kinase, MK2 and hsp27 (Rane et al., 2001), Akt may participate in the regulation of the interaction of p38 with hsp27. It has also been reported that Akt activation is dependent on phosphatidylinositol 3-kinase (PI3K) (Burgering and Coffer, 1995) and PI3K can be activated by arachidonic acid in a variety of cell type (Hii et al., 2001). Thus, arachidonic acid was use to stimulate the L929 cells transfected with both CFP-hsp27 and YFP-p38 fusion protein expressing vectors, and to see if the FRET between these two fusion proteins was affected by activation of Akt. The fluorescence intensity images and lifetime images of CFP in the cell expressing both fusion proteins were taken at various moments after the stimulation by 10 μM arachidonic acid. As sown in Figure 9, the fluorescence lifetime of CFP in the cell increased from 1.69 ns before arachidonic acid-stimulation to 1.9, 2.05 and 2.11 ns at 6, 12 and 18 min respectively after the stimulation, which means that the FRET becomes weaker and weaker after the stimulation. The results may suggest that the activation of Akt leads to loss of the interaction of p38 with hsp27.

In order to further testify the role of Akt in regulating the interaction of p38 with hsp27, wortmannin, an inhibitor of phosphatidylinositol 3-kinase (PI3K) upstream of Akt, was used to inactivate the activation of Akt in arachidonic acid-stimulated cells. The fluorescence intensity images and lifetime images of CFP in the cell expressing the two fusion proteins were taken at various moments after stimulation by arachidonic acid in the presence of 100 nM wortmannin. The results are shown in Figure 10. It is evident that the presence of wortmannin prevents the lifetime of the CFP-hsp27 from being lengthening in the cells expressing both CFP-hsp27 and YFP-p38 after the stimulation. The results suggests that inactivation of Akt by the inhibitor of PI3K also prevent the interaction between p38 and hsp27 from being lost.
Figure 9. The fluorescence intensity images (up panels) and the fluorescent lifetime images (lower panels) of CFP in the L929 cell expressing both the CFP-hsp27 and YFP-p38 fusion proteins before and after stimulation by 10 μM arachidonic acid.

Figure 10. The fluorescence intensity images (up panels) and the fluorescent lifetime images (lower panels) of CFP in the L929 cell expressing both the CFP-hsp27 and YFP-p38 fusion proteins and in the presence of 100 nM wortmannin, the PI3K inhibitor, after stimulation by 10 μM arachidonic acid.

5. DISCUSSION

Although it has been reported that hsp27, p38, Akt and MK2 form a stable complex (Rane et al., 2001), how they interact with each other and how the interaction is regulated are not fully understood. The complex was identified by immunoprecipitation method using anti-Akt, anti-p38, anti-hsp27 and anti-MK2 antibodies. No FRET study on the complex has been performed. In particular, no one has observed the complex and the interaction within the complex in living cells. The present
FRET study provides new information about the complex. First, we observed a fluorescence resonance energy transfer from the hsp27-fused cyan fluorescent protein to the p38-fused yellow fluorescent protein in single living cells. When p38 is activated by \( \text{H}_2\text{O}_2 \)-stimulation, such an energy transfer disappeared. This is consistent with the report that hsp27 dissociates from this complex when cell was stimulated. The question "Whether the dissociation of hsp27 from the complex is due to phosphorylation of hsp27 by p38-activated MK2 or phosphorylation of other components in this complex?" needs to be further investigated. Second, we observed that activation of Akt by activating PI3K signaling leads to loss of the interaction between hsp27 and p38. This could be the consequence of the phosphorylation of hsp27 by Akt on Ser-82 residue (Rane et al., 2003). However, we also observed that either SB203580, the inhibitor of p38, or wortmannin, the inhibitor of PI3K was able to prevent the interaction between p38 and hsp27 from being lost when cell was stimulated either by \( \text{H}_2\text{O}_2 \) or by arachidonic acid. The FRET experiment with these two inhibitors further proves that the activation of either p38 or Akt results in loss of the interaction of p38 with hsp27. This study may well demonstrate the regulatory role of Akt in the interaction of p38 with hsp27.

The study on the interaction of small heat shock protein 27 with MAP kinase p38 by FRET technology described in this chapter may provide a good example for showing the power of FRET technique. FRET imaging microscopy has been proved to be an extremely useful tool in the detection of protein-protein interactions and protein conformational changes in a single living cell. Future application of FRET imaging may involve cellular events coupled to specific molecular signaling processes, imaging in thick tissues or organisms using multiphoton excitation and detection of single-molecule FRET.

6. ACKNOWLEDGEMENTS

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7. REFERENCES


